INVESTIGATIONS ON THE COMPOSITION AND NUTRITIVE VALUE OF VANASPATI. Vol 2

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INVESTIGATIONS ON THE COMPOSITION AND NUTRITIVE VALUE OF VANASPATI

VOLUME TWO

A COLLECTED ACCOUNT OF THE RESEARCHES SPONSORED BY THE CSIR VANASPATI RESEARCH ADVISORY COMMITTEE, 1952-57

COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
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INTRODUCTION

In 1952, the Council of Scientific & Industrial Research, with the concurrence of the Ministry of Food & Agriculture, brought out a publication entitled *Investigations on the Composition and Nutritive Value of Vanaspati*. That publication represented a compilation of the results of researches carried out under the auspices of the former Food Department of the Government of India (now merged with the Ministry of Food & Agriculture) and the Council of Scientific & Industrial Research. The present volume, which is the second in the series, relates exclusively to the results of investigations sponsored by the *Vanaspati Research Advisory Committee* of the Council of Scientific & Industrial Research.

The vanaspati industry, though comparatively young, has made phenomenal progress during the past two and a half decades of active existence. The progress was seriously threatened in the late forties consequent on the reports that the product was injurious to health. The Government under the then Minister for Food & Agriculture (Dr. Rajendra Prasad, now the distinguished President of India) kept an open mind and provided adequate funds and facilities for a fairly exhaustive investigation, on a planned collaboration basis, at a number of leading institutions in the country. The investigation was designed to throw light on the nutritive value of vanaspati with particular reference to the poor diets as consumed in some parts of the country. A team of over 25 research workers was engaged on the study which extended over a period of 3 years. The results showed conclusively that no deleterious effect is produced by vanaspati with a melting point of 37°C. The investigation, though restricted in scope, represents one of the best collaborative efforts of scientists in India. The Government accepted the report of the scientists.

The vanaspati industry, through its Manufacturers' Association, realizing the value of scientific research, generously came forward with the offer of funds to be placed unconditionally at the disposal of the Council of Scientific & Industrial Research for further research on vanaspati. This was accepted by the Governing Body of the Council, which appointed a scientific committee (the *Vanaspati Research Advisory Committee*) exclusively for organizing research for the industry.

Since the publication of the first volume in 1952, the *Vanaspati Research Advisory Committee* has sponsored researches mainly at three centres, namely, the University College of Science & Technology, Calcutta, the Nutrition Research Laboratories, Coonoor, and the Central Food Technological Research Institute, Mysore. A large part of the research work carried out at these centres has already been published in the form of scientific papers. The present publication represents a consoli-

dated account of the results and serves as a reference volume on the progress of vanaspati research for the use of industry, scientists and others interested in the subject.

The Committee has also sponsored the publication of Annual Reviews under the series title: *Literature Survey on Oils and Fats.* These surveys, prepared by the staff attached to the Central Food Technological Research Institute, Mysore, have helped to present in a compact form the more important advances in the field of oils and fats, not only in India, but also in other parts of the world.

The vanaspati industry has on its rolls, quite a number of active scientists engaged in scientific control and testing work, and in the study of problems that face the industry. In addition to these, there is need for both fundamental and applied research of a specialized nature which could be best carried out in universities and research laboratories.

The present publication owes its origin to the initiative and keen interest of Prof. M.S. Thacker, Director-General of Scientific & Industrial Research, and Mr. S.H. Turner (Chairman, *Hindustan Lever Ltd.*), representative of the industry on the *Vanaspati Research Advisory Committee.* To them and to the contributors, who readily responded to the invitation, our thanks are due.

Mysore
May 27, 1958

V. SUBRAHMANYAN

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Studies on the Stability of Hydrogenated Oils and of Vitamin A in Groundnut Oils and on the Physiological Suitability of Ethyl Gallate as an Antioxidant

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1. STABILITY OF HYDROGENATED GROUNDNUT OILS

Studies on the stability of hydrogenated oils are of importance to the vanaspati industry. Results of tests on the stability of groundnut oils (raw, refined and hydrogenated) under various conditions have been reported in a previous publication[1]. The studies have been extended to cover stability of hydrogenated groundnut oils blended with various oils, crude, refined and hydrogenated, in comparison with straight-hardened groundnut oil. The results of this study are presented below.

Blended and Straight-hardened Oils

The stability of hydrogenated groundnut oil of different melting points blended with varying proportions of sesame oil (crude and refined) has been studied and compared with the stability of straight-hardened groundnut oil[2,3].

The composition and characteristics of three samples of blended oils and one of straight-hardened groundnut oil used in this study, are given in Table 1. Samples of blends and straight-hardened oil were kept in sealed tins and also in loose-cover tins at 37°C. One sealed tin and one loose-cover tin were taken out every month for determining peroxide value (ml. of N/500 thiosulphate per g. of oil) and acid and iodine values. Linoleic acid was estimted by the spectrophotometric method of Hilditch *et al.*[4], the oleic acid content was calculated from the iodine value and the linoleic acid content, as no other unsaturated acids are present in the oils studied.

At the end of 5 months, no rancid odour could be detected in any of the samples stored in closed tins, but rancidity was detected, from the second month onwards, in blended samples stored in loose-cover tins.

Among the blended hydrogenated groundnut oil samples (all melting at 37°C.), the ones having a greater proportion of refined groundnut oil showed higher rates of peroxide and acid formation (Tables 1 and 2).

TABLE 1—CHARACTERISTICS OF BLENDED AND STRAIGHT-HARDENED HYDROGENATED GROUNDNUT OILS

Sample No.	% Composition	m.p. °C.	Iod. val.	Peroxide val.*	Acid val.	Fatty acids, %		
						Linoleic	Oleic	Saturated
1	Hydrogenated oil (m.p. 41°C.), 40 ; sesame oil, 5 ; refined oil, 55	37.0	80.1	3.0	0.12	13.6	61.5	24.9
2	Hydrogenated oil (m.p. 50.8°C.), 10 ; sesame oil, 5 ; refined oil, 85	36.0	88.0	3.2	0.17	21.1	55.2	23.7
3	Hydrogenated oil (m.p. 61.0°C.), 5 ; sesame oil, 5 ; refined oil, 90	36.0	90.3	6.8	0.16	23.2	53.3	23.3
4	Straight-hardened oil containing 5% sesame oil	37.0	69.2	0.0	0.11	1.7	73.0	25.3

*ml. of N/500 thio./g. fat

TABLE 2—CHARACTERISTICS OF BLENDED AND STRAIGHT-HARDENED OILS STORED IN TINS

Sample* No.	Closed tin							Loose cover tin					
	Peroxide val.†			Acid val.		Iod. val.		Peroxide val.†		Acid val.		Iod. val.	
	Initial	After 3 months	After 5 months	Initial	After 5 months	Initial	After 4 months	Initial	After 4 months	Initial	After 4 months	Initial	After 4 months
1	3.0	7.1	4.1	0.12	0.26	80.1	76.0	3.0	21.9	0.12	0.21	80.1	75.5
2	3.0	7.2	3.9	0.17	0.29	88.0	83.8	3.2	35.9	0.17	0.51	88.0	82.9
3	6.8	8.0	4.9	0.16	0.27	90.3	85.1	6.8	40.7	0.16	0.26	90.3	85.0
4	0.0	1.1	1.6	0.11	0.18	69.0	65.7	0.0	2.1	0.11	0.16	69.0	65.0

* Composition same as given in Table 1

† ml. of N/500 thio./g. fat

The straight-hardened hydrogenated groundnut oil appeared to be more stable (indicated by peroxide and acid values) than even the blended sample containing the least amount of refined oil. The stability of the samples (judged by peroxide and acid values) was roughly inversely proportional to the linoleic acid content. Oleic acid, unlike linoleic acid, did not influence the stability adversely, as it was observed that the straight-hardened sample having the highest proportion of oleic acid had the greatest stability. It is likely that oleic acid does not play any significant part so long as linoleic acid is present.

In view of the fact that highly hardened fat can be blended with a liquid oil in suitable proportions to conform to the consistency of straight-hardened fat, the determination of linoleic acid content, and, to a less extent, of iodine value would help to differentiate blended vanaspati containing liquid oil from straight-hardened vanaspati. It may, however, be noted that while the blended vanaspati is less stable than the straight-hardened sample, it contains more linoleic acid which may be of nutritional significance.

The increase in acid value was usually small. Though the acid value has probably no direct relation to peroxidation, the rate of increase of acid value was roughly proportional to that of peroxide value. Antioxidants usually suppressed acid formation slightly, and peroxide formation considerably.

The peroxide value of blended samples stored in sealed tins rose initially, but decreased after 2 to 3 months.

Vanaspati Containing Other Hydrogenated Oils

Considering the fact that vegetable oils other than groundnut oil may also be used for the manufacture of vanaspati, it is useful to study the stability of vanaspati made from red palm, linseed, mustard and cottonseed oils. The results of investigation on stability of vanaspati made from varying proportions of these oils and that of hydrogenated groundnut oil[3] are summarised below.

Hydrogenated groundnut oil containing 5 per cent sesame oil and 0,10,20,30,40 and 50 per cent hydrogenated palm oil, and hydrogenated linseed and mustard oils containing 5 per cent sesame oil and 0,20,45 and 70 per cent hydrogenated groundnut oil were used in this investigation. The characteristics of the samples are given in Table 3.

The samples were packed in 5-lb. sealed tins and kept at room temperature (15°-31° in the case of linseed oil and 15°-40° in the case of palm and mustard oil samples). The results are given in Table 4. In another set of experiments, 100 g. samples were stored at 37° with and without ethyl gallate (0.01 per cent) and with added vitamin A (30 i.u. per g. of sample). Samples were withdrawn every month for examination. Vitamin A was determined colorimetrically by the Carr-Price reaction[5]. The results are given in Tables 5, 6 and 7.

No rancid odour was observed in closed tin samples; nor was there any appreciable rancid odour in loose-cover tin samples.

There was hardly any difference in peroxide or acid values among the samples containing different proportions of mustard oil stored in sealed tins. In linseed oil samples, on the other hand, the peroxide value rose markedly with increasing proportion of hydrogenated linseed oil; the acid value also increased, though not so markedly. In samples containing

TABLE 3—CHARACTERISTICS OF VANASPATI CONTAINING HYDROGENATED PALM, MUSTARD AND LINSEED OILS

Sample No.	Hardened oil added	% Composition of vanaspati			m.p. °C.	Iod. val.	Sap. val.	Peroxide* val.	Acid val.
		Hardened oil	Hardened groundnut oil	Sesame oil					
1	Palm	0	95	5	37.0	70.6	188.0	0.3	0.06
2		10	85	5	37.0	68.0	188.9	0.3	0.10
3		20	75	5	37.0	64.7	189.7	0.3	0.20
4		30	65	5	37.0	63.4	190.6	0.3	0.19
5		40	55	5	37.0	61.2	191.4	0.3	0.22
6		50	45	5	37.0	60.0	192.3	0.4	0.22
a	Mustard	25	70	5	36.8	71.7	185.8	0.2	0.07
b		50	45	5	37.0	72.1	179.8	0.1	0.08
c		75	20	5	37.2	73.7	175.1	0.6	0.09
d		95	..	5	37.0	77.7	171.0	0.5	0.08
I	Linseed	25	70	5	37.0	75.9	190.5	0.2	0.02
II		50	45	5	37.0	81.1	190.0	0.3	0.03
III		75	20	5	37.0	86.3	189.6	0.4	0.02
IV		95	..	5	37.0	91.3	189.1	0.4	0.08

* ml. of N/500 thio./g. fat

TABLE 4—CHARACTERISTICS OF VANASPATI CONTAINING DIFFERENT HYDROGENATED OILS STORED IN SEALED TINS

Sample* No.	Hardened oil added	Peroxide val.†		Acid val.	
		Initial	After 5 months	Initial	After 5 months
1	Palm	0.3	2.5	0.06	0.16
2		0.3	2.8	0.10	0.23
3		0.3	2.6	0.20	0.29
4		0.3	2.6	0.19	0.34
5		0.3	2.6	0.22	0.42
6		0.4	2.6	0.22	0.45
a	Mustard	0.2	0.8	0.07	0.08
b		0.1	0.8	0.08	0.09
c		0.6	0.9	0.09	0.09
d		0.5	0.8	0.08	0.09
I	Linseed	0.2	1.4	0.02	0.14
II		0.3	1.7	0.03	0.16
III		0.4	2.3	0.02	0.16
IV		0.4	2.9	0.08	0.18

* Composition as given in Table 3
† ml. of N/500 thio./g. fat

increasing proportions of palm oil, the rise was marked in acid value, but not in peroxide value.

In loose-cover tins also, higher proportions of linseed and mustard oils induced more peroxide formation. In the case of palm oil, however, a higher proportion of it gave a lower peroxide value owing apparently to the lower iodine value of palm oil. The increase in acid value in all samples was small, but higher proportions of all the three oils increased the acid value.

Ethyl gallate suppressed peroxide formation markedly ; it had only slight effect on acid value. Vitamin A was more stable in samples containing palm oil than in samples containing linseed or mustard oil. The stability of vitamin A appeared to be related to peroxide formation in different samples.

Effect of Sesame Oil on Stability and Properties of Vanaspati

It had been found earlier in this laboratory that crude sesame oil stabilised vanaspati better than refined sesame oil[6]. This investigation

TABLE 5—STABILITY OF VANASPATI CONTAINING HYDROGENATED PALM OIL STORED IN LOOSE-COVER TINS

Sample* No.	Additive	Peroxide val.†		Acid val.		Vitamin A content, *i.u./g.*	
		Initial	After 5 months	Initial	After 5 months	Initial	After 5 months
1	Nil	0.3	7.1	0.06	0.19	..	..
	E.G.	0.3	3.7	0.06	0.18	..	..
	Vitamin A	0.3	7.3	0.06	0.18	30.0	17.3
	E.G.+vitamin A	0.3	3.7	0.06	0.18	30.0	18.2
2	Nil	0.3	7.1	0.10	0.31	..	..
	E.G.	0.3	4.9	0.10	0.29	..	..
	Vitamin A	0.3	6.9	0.10	0.29	30.0	17.4
	E.G.+vitamin A	0.3	4.7	0.10	0.26	30.0	16.3
3	Nil	0.3	5.9	0.20	0.34	..	..
	E.G.	0.3	5.6	0.20	0.37	..	..
	Vitamin A	0.3	7.0	0.20	0.36	30.0	17.4
	E.G.+vitamin A	0.3	4.4	0.20	0.36	30.0	16.3
4	Nil	0.3	5.8	0.19	0.44	..	..
	E.G.	0.3	3.5	0.19	0.39	..	..
	Vitamin A	0.3	6.7	0.19	0.39	30.0	21.1
	E.G.+vitamin A	0.3	3.5	0.22	0.41	30.0	21.0
5	Nil	0.3	6.6	0.22	0.48	..	..
	E.G.	0.3	5.9	0.22	0.48	..	..
	Vitamin A	0.3	5.6	0.22	0.52	30.0	20.0
	E.G.+vitamin A	0.3	4.0	0.22	0.45	30.0	20.0
6	Nil	0.4	6.3	0.22	0.50	..	..
	E.G.	0.4	3.7	0.22	0.29	..	..
	Vitamin A	0.4	7.4	0.22	0.45	30.0	20.0
	E.G.+vitamin A	0.4	3.9	0.22	0.52	30.0	21.2

* Composition same as given in Table 3, E.G.—Ethyl gallate
† ml. N/500 thio./g. fat

TABLE 6—STABILITY OF VANASPATI CONTAINING HYDROGENATED MUSTARD OIL STORED IN LOOSE-COVER TINS

Sample* No.	Additive	Peroxide val.†		Acid val.		Vitamin A *i.u./g.*	
		Initial	After 5 months	Initial	After 5 months	Initial	After 5 months
a	Nil	0.2	..	0.07	..	..	..
	E.G.	0.2	1.8	0.07	0.09	..	..
	Vitamin A	0.2	5.4	0.07	0.08	30	18.0
	E.G.+vitamin A	0.2	1.4	0.07	0.08	30	22.5
b	Nil	0.1	7.3	0.08	0.10	..	..
	E.G.	0.1	1.9	0.08	0.09	..	..
	Vitamin A	0.1	9.7	0.08	0.10	30	15.3
	E.G.+vitamin A	0.1	1.9	0.08	0.08	30	20.9
c	Nil	0.6	17.8	0.09	0.12	..	..
	E.G.	0.6	4.5	0.09	0.10	..	..
	Vitamin A	0.6	15.5	0.09	0.15	30	14.3
	E.G.+vitamin A	0.6	3.1	0.09	0.11	30	17.6
d	Nil	0.5	14.4	0.08	0.12	..	..
	E.G.	0.5	4.1	0.08	0.10	..	..
	Vitamin A	0.5	15.3	0.08	0.11	30	15.8
	E.G.+vitamin A	0.5	3.2	0.08	0.11	30	17.6

* Composition as given in Table 3. E.G.—Ethyl gallate

† ml. N/500 thio./g. fat

TABLE 7—STABILITY OF VANASPATI CONTAINING HYDROGENATED LINSEED OIL STORED IN LOOSE-COVER TINS

Sample* No.	Additive	Peroxide val.†		Acid val.		Vitamin A *i.u. g.*	
		Initial	After 5 months	Initial	After 5 months	Initial	After 5 months
I	Nil	0.2	7.2	0.02	0.14	..	..
	E.G.	0.2	1.5	0.02	0.14	..	..

Contd.

Table 7—*Contd.*

	Vitamin A	0.2	6.7	0.02	0.14	30.0	14.5
	E.G.+vitamin A	0.2	1.4	0.02	0.14	30.0	18.0
II	Nil	0.3	8.7	0.03	0.17	..	..
	E.G.	0.3	1.5	0.03	0.16	..	..
	Vitamin A	0.3	8.7	0.03	0.18	30.0	14.5
	E.G.+vitamin A	0.3	1.6	0.03	0.16	30.0	16.5
III	Nil	0.4	9.0	0.02	0.18	..	..
	E.G.	0.4	2.3	0.02	0.17	..	..
	Vitamin A	0.4	9.2	0.02	0.17	30.0	14.5
	E.G.+vitamin A	0.4	3.2	0.02	0.17	30.0	16.0
IV	Nil	0.4	9.6	0.08	0.22	..	..
	E.G.	0.4	..	0.08	..	..	..
	Vitamin A	0.4	8.5	0.08	0.23	30.0	13.0
	E.G.+vitamin A	0.4	2.3	0.08	0.21	30.0	13.0

*Composition as given in Table 3. E.G.—Ethyl gallate †ml. N/500 thio. g. fat

Table 8—Characteristics of Crude, Refined and Hydrogenated Sesame Oils and Hydrogenated Groundnut Oil

Oil	Iod. val.	Peroxide* val.	Acid val.	Sap. val.	Baudouin test†
Crude sesame	111.8	1.7	2.70	190.5	149.5
Refined sesame	104.3	3.7	0.27	188.1	95.0
Hydrogenated sesame (m.p. 37°C.)	84.7	0.0	0.23	187.0	90.0
Hydrogenated groundnut (m.p. 37°C.)	66.6	0.0	0.27	192.0	..

*ml. N/500 thio./g. fat ‡Lovibond Red units, 1 cm.cell

was continued in order to study the effect of raw sesame oil addition on flavour, colour and taste of vanaspati under storage and the change during storage in the intensities of Baudouin test.

The characteristics of crude, refined and hydrogenated sesame oils and hydrogenated groundnut oil used in the study are given in Table 8. Portions (100 g.) of hydrogenated groundnut oil containing 5 per cent sesame oil (crude, refined and hydrogenated), with and without ethyl gallate (0.01 per cent) were stored in 1 lb. tins at 37°C. Samples were taken out at fortnightly intervals and examined for Baudouin test and other characteristics (Table 9).

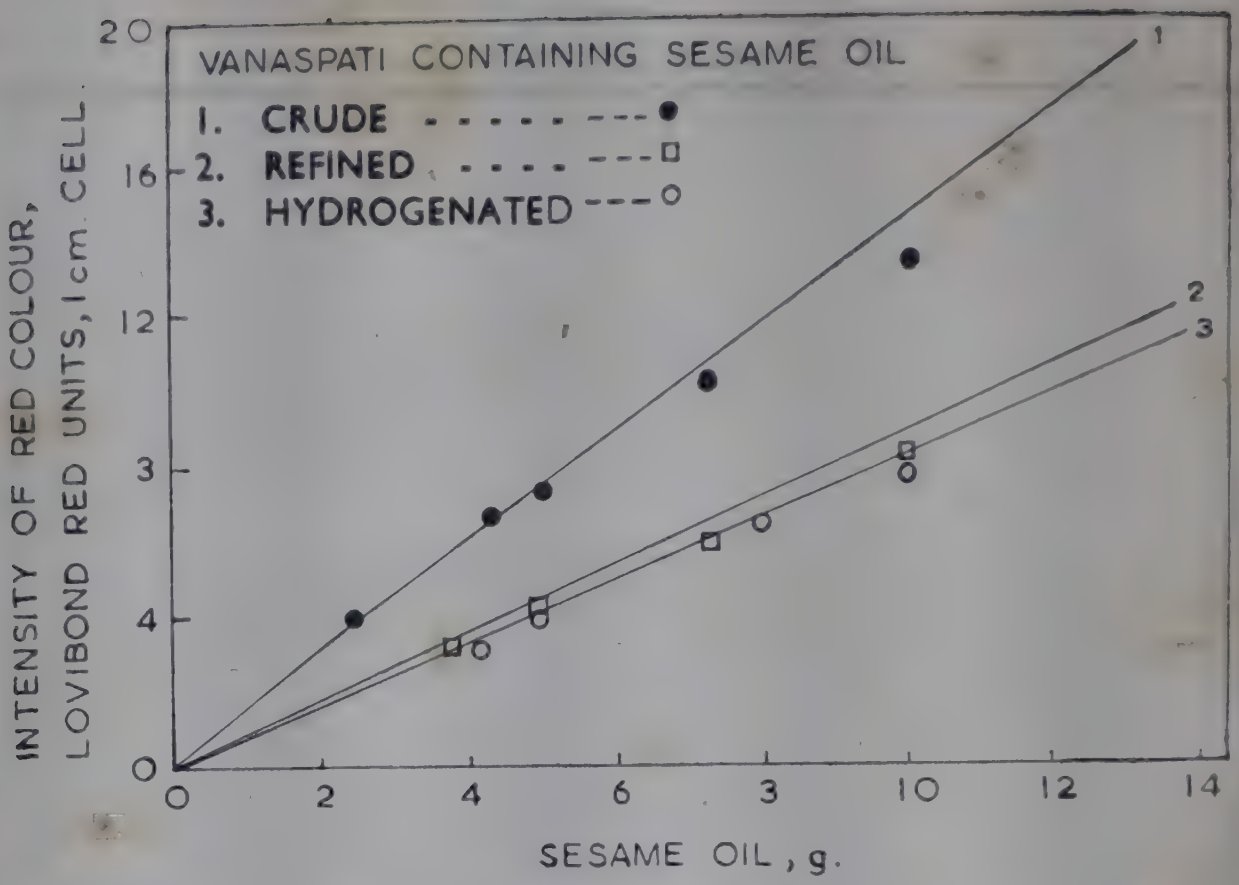

FIG. 1—COLOUR INTENSITY OF FATS (IN BAUDOUIN TEST) WITH DIFFERENT PERCENTAGES OF SESAME OIL

Fig. 1 shows that there is a linear relation within certain limits, between the amount of sesame oil taken and the intensity of red colour (in Baudouin test) of the aqueous layer which has a fixed volume. The linearity, however, begins to deviate with increasing proportions of the oil.

It will be observed from Table 9 that crude sesame oil is superior to refined sesame oil or hydrogenated sesame oil for incorporation in vanaspati. The intensity of colour with crude oil in the Baudouin test is consistently greater and persists for longer periods of storage. Further, there is no appreciable difference in taste, flavour and colour of vanaspati samples containing crude, refined and hydrogenated sesame oil, so that the use of crude sesame oil in vanaspati appears to be preferable.

2. STABILITY OF ADDED VITAMIN A IN GROUNDNUT OILS

Vanaspati produced in India is enriched with 700 i.u. of synthetic vitamin A per oz. as per the *Vanaspati Control Order* of the Government of India. Studies on the stability of vitamin A are of importance to the industry and a few papers on this subject[8–10] have been published. The stability of vitamin A acetate and Vanitin (*Hoffman-La-Roche*) added to crude and refined groundnut oils and blended and straight-hardened groundnut oils has been studied under different storage conditions and also when heated and used as a cooking medium[8–11]. A summary of the results obtained is presented below.

Stability of Vitamin A Acetate in Groundnut Oils

Stability on storage: Crude groundnut oil, refined groundnut oil, hydrogenated groundnut oil, m.p. 37°C., blended hydrogenated ground-

TABLE 9—CHARACTERISTICS OF VANASPATI CONTAINING SESAME OILS STORED WITH AND WITHOUT ETHYL GALLATE

Oil	Peroxide val.*		Baudouin test†		Rancidity (odour)		Flavour, colour & taste	
	Initial	After 14 weeks	Initial	After 14 weeks	Initial	After 14 weeks	Initial	After 14 weeks
Crude	0.1	2.1	7.5	1.0	Fresh	Fresh	Good	No appreciable change
Refined	0.2	2.5	4.7	0.8	do	do	do	do
Hydrogenated	0.0	2.1	4.5	0.0	do	do	do	do
Crude+E.G.	0.1	1.4	7.5	1.2	do	do	do	do
Refined+E.G.	0.2	1.4	4.7	0.7	do	do	do	do
Hydrogenated+E.G.	0.0	1.0	4.5	0.0	do	do	do	do

E.G.—Ethyl gallate
*ml. N/500 thio./g. fat
†Lovibond Red Units, 1 cm. cell

Table 10—Characteristics of Groundnut Oils

Oil	Peroxide val.*	Acid val.	Sap. val.	Iod. val.
Crude	2.4	2.02	193.0	91.35
Refined	4.0	0.82	195.5	91.94
Straight-hardened	0.1	0.21	185.4	61.46
Blended	2.9	0.30	189.6	76.25

*ml. N/500 thio./g. fat

nut oil, m.p. 37°C. 1 part of hydrogenated oil, m.p. 55°C. and 5 parts of refined groundnut oil) and straight-hardened groundnut oil, m.p. 37°C. have been used in these studies. Ethyl gallate was used as antioxidant.

The initial characteristics of the oils are given in Table 10. The percentage of vitamin A; peroxide values; and rancidity of oils after 21 days storage at 60°C. and 59 days storage at 37.5°C. have been determined and the effect of addition of ethyl gallate (0.1 per cent) on these properties has been studied. For comparison, the corresponding values, when liquid paraffin (an inert non-autoxidising vehicle) is added in place of ethyl gallate, have been determined. The results are presented in Table 11.

The results (Table 11) show that straight-hardened oil retained the maximum amount of vitamin A both at 37·5° and 60°C. while the refined oil retained the least amount. The retention of vitamin A was slightly better in crude oil than in blended oil. Ethyl gallate afforded significant protection to vitamin A acetate in all samples, though the degree of protection was small. Vitamin A acetate appeared to promote slight instability of oils, which, however, was counteracted to an appreciable extent by ethyl gallate. Rancidity (as detected by smell) was, however, not promoted appreciably by vitamin A acetate.

Effect of adding sesame oil: As it is compulsory to add sesame oil to vanaspati, it was of interest to examine the effect of adding sesame oil (crude, refined and hydrogenated) on the stability of added vitamin A in vanaspati.

The initial characteristics of the oil samples and the values obtained after storage for 14 weeks at 37°C. are given in Tables 12 and 13 respectively.

Addition of crude and hydrogenated sesame oil stabilises vitamin A almost to the same extent (Table 13) ; refined sesame oil, however, is not affected. As observed earlier, vitamin A itself tends to increase the peroxide value of oils, which is counteracted to some extent by ethyl gallate.

Stability of vitamin A during heating and cooking: One hundred grams of hydrogenated groundnut oil (m.p. 37°C.), containing 30 i. u. of vitamin A acetate per g., with and without ethyl gallate (0·01 per cent), was heated (with periodic gentle shaking) in an open pyrex beaker on an oil bath at different temperatures and for different periods. Samples were withdrawn at intervals, cooled rapidly and analysed for vitamin A (Table 14). Cooking experiments were carried out in a small iron pan ; the oil (with and without ethyl gallate) was used as a cooking medium for preparing three Indian dishes and the vitamin contents of the preparations and the oil left over have been determined (Table 15).

TABLE 11—STABILITY OF VITAMIN A ACETATE IN GROUNDNUT OILS WITH AND WITHOUT ETHYL GALLATE

Oil	Vitamin A acetate (i.u./g.)			Peroxide val.*			Rancidity (odour)†	
	Initial	After 21 days at 60°	After 59 days at 37.5°	Initial	After 21 days at 60°	After 59 days at 37.5°	After 21 days at 60°	After 59 days at 37.5°
Crude oil	100	0.0	5.1	2.4	32.9	30.9	R	R
do +E.G.	100	14.4	8.6	2.4	28.4	12.9	R	R
do	30	0.0	0.0	2.4	37.8	27.7	R	R
do +E.G.	30	9.2	3.5	2.4	32.2	8.8	S	V.S.
Refined oil	100	0.0	1.7	4.0	30.8	49.0	R	R
do +E.G.	100	5.1	7.0	4.0	15.4	6.6	R	R
do	30	0.0	0.0	4.0	118.3	48.6	R	R
do +E.G.	30	0.0	3.5	4.0	57.1	26.2	S	R
Straight-hardened oil	100	10.4	17.8	0.1	6.9	2.8	V.S	V.S
do +E.G.	100	12.1	17.8	0.1	4.2	1.5	F	F
do	30	8.6	10.2	0.1	3.5	3.2	V.S	V.S
do +E.G.	30	3.5	13.5	0.1	0.1	1.4	V.S	F
Blended oil	100	0.0	1.7	2.9	58.8	33.6	S	V.S
do + E.G.	100	10.5	8.6	2.9	8.9	9.8	V.S	V.S
do	30	0.0	0.0	2.9	153.3	25.9	S	S
do + E.G.	30	6.3	7.0	2.9	13.7	8.7	V.S	V.S
Liquid paraffin	100	20.3	33.0	0.0	0.0	0.0	..	..
do + E.G.	100	21.1	37.2	0.0	0.1	0.0	..	..
do	30	8.7	12.2	0.0	0.0	0.0	..	..
do + E.G.	30	9.0	14.1	0.0	0.0	0.1	..	..

E.G.—ethyl gallate *ml. N/500 thio. g. fat

† V.S.—very slight rancidity ; S—slight rancidity ; R—appreciable rancidity ; F—aroma of fresh oil

Table 12—Characteristics of Sesame Oils

Oil	Iod. val.	Peroxide val.*	Acid val.	Sap. val.
Crude sesame	111.8	1.7	2.70	190.5
Refined	104.3	3.7	0.27	188.1
Hydrogenated, m.p. 37°	84.7	0.0	0.23	187.0
Hydrogenated groundnut, m.p. 37°	66.6	0.0	0.27	192.0

*ml. N/500 thio./g. fat

Table 13—Stability of Vitamin A in Vanaspati with added Sesame Oil

Additive	Peroxide val.*		Vitamin A (*i.u./g.*)		Rancidity after 14 weeks
	Initial	After 14 weeks	Initial	After 14 weeks	
Crude oil	0.1	3.1	30	12	nil
do +E.G.	0.1	2.5	30	16	slight
Refined oil	0.2	4.2	30	7	do
do + E.G.	0.2	2.8	30	11	do
Hardened oil, m.p. 37°	nil	2.5	30	11	nil
do + E.G.	nil	2.0	30	16	slight

E.G.—Ethyl gallate *ml. N/500 thio./g. fat

Table 14—Effect of Heating on Vitamin A in Vanaspati

(*Initial conc. of vitamin A, 30 i.u./g.*)

Heating time *min.*	Temp. °C.	Vitamin A after heating *i.u./g.*		Loss, %	
		Without E.G.	With E.G.	Without E.G.	With E.G.
2	110	28	30	6.7	0.0
5	110	20	28	33.3	6.7
2	150	20	27	33.3	10.0
5	150	15	21	50.0	30.0
2	200	17	24	42.3	20.0
5	200	10	14	66.7	52.3
2	250	9	15	70.0	50.0
5	250	0	9	100.0	70.0

E.G.—Ethyl gallate

TABLE 15—LOSS OF VITAMIN A DURING COOKING

Prepared food	Initial temp. C°.	Vitamin A before cooking *i.u./g.*		Vitamin A in prepared food *i.u./g.*		Vitamin A in cooked oil *i.u./g.*		Loss, %	
		Without E.G.	With E.G.	Without E.G.	With E.G.	Without E.G.	With E.G.	Without E.G.	With E.G.
*Luchi**	200	9000	9000	1360	1570	6910	7550	19.0	10.5
Halua†	200	900	900	640	780	..	..	29.0	13.3
Potato chips‡	200	900	900	320	375	400	440	20.0	9.5

* Flour + water + oil (5 : 2 : 1) made into dough; 25 g. made into discs of 4 in. diam.
† Suji, 30 g. + sugar, 25 g. + oil, 30 g. + water
‡ Slices (1 in. × 1 in. × 1/16 in.); 30 g.
E.G.—Ethyl gallate

TABLE 16—VITAMIN A CONTENT PEROXIDE AND ACID VALUES OF VANASPATI AFTER 3 MONTHS' STORAGE

(*Vanaspati, m.p., 37°; iod. val., 66.3; acid val., 0.30; peroxide value, 0*)

		Vit. A *i.u./g.*	Peroxide val.*	Acid val.
Hydrogenated groundnut oil		..	5.9	0.42
do	+ 10.6 i.u./g. of vit. A	2	6.2	0.43
do	+ do + E.G., 0.01%	2	2.0	0.41
do	+ 30 i.u./g. of vit. A	12	7.1	0.43
do	+ do + E.G., 0.01%	12	1.9	0.42
do	+ 100 i.u./g. of vit. A	49	7.3	0.47
do	+ do + E.G., 0.01%	50	1.7	0.44

* ml. N/500 thio./g. fat E.G.—Ethyl gallate

It was observed (Table 14) that the longer the duration of heating and the higher the temperature, the greater was the destruction of vitamin A. Ethyl gallate afforded protection to vitamin A during both heating and cooking.

Stability of Vanitin in Groundnut Oils

Stability on storage: Enriched vanaspati was stored in loose-cover tins for 3 months at 37°C.; ethyl gallate was then added (0·01 per cent) as antioxidant. The vitamin A content was determined after a fortnight and again after 2 months. It was found (Table 16) that the

TABLE 17—VITAMIN A CONTENT PEROXIDE AND ACID VALUES OF VANASPATI AFTER 5 MONTHS' STORAGE

(*Vanaspati: iod. val., 64.5 ; peroxide val., 0.7 ; acid val., 031 ; sap. val., 191.2, m.p., 37°C.*)

	Vitamin A *i.u./g.*		Peroxide value*		Acid value	
	Initial	After storage	Initial	After storage	Initial	After storage
Vanaspati	..	..	0.7	5.1	0.31	0.44
do + Vanitin						
(*a*)	30.0	9.9	0.7	4.6	0.31	0.41
(*b*)	10.6	4.5	0.7	4.6	0.31	0.40
Vanaspati + Vanitin + E.G.						
(*a*)	30.0	10.0	0.7	1.4	0.31	0.46
(*b*)	10.6	5.0	0.7	1.4	0.31	0.45
Vanaspati + Vanitin + P.G.						
(*a*)	30.0	9.8	0.7	1.4	0.31	0.56
(*b*)	10.6	4.5	0.7	0.9	0.31	0.52
Vanaspati + Vanitin + NDGA						
(*a*)	30.0	13.6	0.7	1.0	0.31	0.43
(*b*)	10.6	4.1	0.7	1.1	0.31	0.39
Vanaspati + Vanitin + Tenox II						
(*a*)	30.0	10.3	0.7	3.2	0.31	0.47
(*b*)	10.6	3.7	0.7	4.3	0.31	0.42
Vanaspati + Vanitin + BHA						
(*a*)	30.0	10.5	0.7	4.3	0.31	0.48
(*b*)	10.6	5.0	0.7	4.6	0.31	0.46

E.G.—Ethyl gallate ; P.G.—Propyl gallate; BHA—Butylated hydroxy anisole ; NDGA—Nordihydroguairetic acid

rate of destruction of vitamin A slowed down after a fortnight. Ethyl gallate gave no material protection, perhaps because Vanitin already contained some antioxidant. None of the samples had a rancid odour after three months' storage period.

Effect of different antioxidants: The effect of adding different antioxidants along with Vanitin was studied. Vanaspati containing Vanitin and antioxidant was stored for 5 months in loose-cover tins. The vitamin A, peroxide and acid value after the storage period are given in Table 17. It will be seen from the results, that the rate of destruction

TABLE 18—STABILITY OF VANITIN AND VITAMIN A ACETATE IN VANASPATI AFTER 5 MONTHS' STORAGE

(*Vanaspati: iod val., 69.0; peroxide val., 0.1; acid val., 0.05; sap. val., 191.0; m.p., 37°C.; sesame oil, 5%; temp. of storage, 37°; Initial conc. of vitamin A, 30 i.u./g.; Initial peroxide val., 0.1*)

Additive	Vitamin A (i.u./g.) after storage	Peroxide value* after storage
Vanitin	25	0.4
do + BHA (0.01%)	26	0.3
do + E.G. (0.01%)	26	0.3
Vitamin A acetate	23	0.7
do + BHA (0.01%)	26	0.3
do + E.G. (0.01%)	25	0.4

E.G.—Ethyl gallate; BHA—Butylated hydroxy anisole
* ml. N/500 thio./g. fat

TABLE 19—EFFECT OF HEATING ON VANASPATI CONTAINING VANITIN

(*Vitamin A from Vanitin, 10.6 i.u./g.*)

Antioxidant	Time of heating *min.*	Temp. °C.	Vitamin A after heating *i.u./g.*	Loss %
nil	2	110	10	5.6
E.G.	2	110	10	5.6
nil	5	110	8	24.5
E.G.	5	110	8	24.5
nil	2	150	8	24.5
E.G.	2	150	9	15.1
nil	5	150	6	43.4
E.G.	5	150	6	43.4
nil	2	200	7	33.9
E.G.	2	200	7	33.9
nil	5	200	5	52.8
E.G.	5	200	6	43.4
nil	2	250	5	52.8
E.G.	2	250	7	33.9
nil	5	250	0	100.0
E.G.	5	250	2	81.1

E.G.—Ethyl gallate

TABLE 20—LOSS OF VITAMIN A DURING COOKING

(Figures of vitamin loss in i.u./g.; cooking medium—vanaspati enriched with Vanitin)

Prepared food	Initial temp. °C.	Cooking temp. °C.	Vitamin A before cooking		Vitamin A in food		Vitamin A in oil		Loss	
			Without BHA	With BHA	Without BHA	With BHA	Without BHA	With BHA	Without BHA	With BHA
*Luchi**	200	165–180	7208	6908	3558	3527	1247	1248	33.4	30.9
Cake*	180	180	3054	4054	2440	2610			20.1	14.5
Halua†	180	145–170	2160	2160	1619	1688			25.0	21.9
Parota†	180	153–168	232	232	196	206			15.8	11.4

*Vanaspati: iod. val., 70.6; peroxide val., 0.5; acid val., 0.23; sap. val. 188.0; and sesame oil, 5%. †Vanaspati: iod. val., 69.0; peroxide val., 0.5; acid val., 0.20; sap. val., 191.0; and sesame oil, 5%

BHA—Butylated hydroxy anisole

TABLE 21—VITAMIN A CONTENT IN COOKING MEDIUM AT DIFFERENT STAGES OF FRYING LUCHI

	Without BHA	With BHA
Before frying	29.3	28.0
After first batch	19.5	23.0
After second batch	15.6	17.4
After third batch	11.6	11.6

BHA—Butylated hydroxy anisole

of vitamin A was the greatest during the first month of storage, after which the rate gradually fell off. None of the antioxidants employed, except NDGA, helped appreciably in protecting vitamin A ; the protection afforded by NDGA was also slight. Apparently the antioxidants present in Vanitin could not be reinforced appreciably by the added antioxidants in loose-cover tins. In regard to peroxide value, ethyl and propyl gallates and NDGA appeared to be somewhat more effective than BHA and Tenox II. None of the samples showed a rancid odour after 5 months' storage.

Stability on storage in sealed tins: The methods of preparing the samples and of canning, given in a previous publication[11] were followed. The results (Table 18) show that the destruction of vitamin A was the greatest during the first month of storage, and, thereafter, the rate of destruction gradually diminished. The protective action of ethyl gallate and BHA was marked during the earlier period of storage. A general protective effect of the antioxidants was observed all through. The loss of vitamin A observed after the first month included what probably occurred during the process of canning. In general, vitamin A of Vanitin was protected better than vitamin A acetate under the conditions studied.

Stability during heating and cooking: Vanaspati containing Vanitin was heated in a pyrex beaker with shaking (but without stirring), at a temperature of 110°C. It was found that heating for a short time did not entail any significant loss of vitamin A (Table 19); heating at high temperatures and for longer periods of time, resulted in considerable loss of Vanitin. Ethyl gallate afforded some protection, but the effect was not constant.

Four common food preparations—*Luchi*, cake, *Halua* and *Parota* were prepared and the vitamin A content of the medium was determined before and after cooking.

The results obtained showed (Tables 20 and 21) that there was appreciable loss of vitamin A, particularly when the same vanaspati was used for preparing successive batches of food. BHA offered, however, some protection.

3. PHYSIOLOGICAL SUITABILITY OF ETHYL GALLATE AS AN ANTIOXIDANT

Since ethyl gallate appeared to be a suitable antioxidant from the standpoint of stability, a few experiments were carried out on the physiological suitability of ethyl gallate. The methods followed in the studies

and the results obtained have already been published[12-16]. The results have been summarised below:

Acute toxicity dose: To cover a wide range of possible variations in the doses, a large number of small groups of rats were used at different dose levels. The acute oral toxicity dose was determined by estimating the dose at which 50 per cent of rats receiving the dose do not survive. This end point is less affected by small variations[17] than any other.

Albino rats (100-200 g.) were fasted for 18 hr. (water, *ad lib*) and each one placed in a separate cage. Samples of ethyl gallate were mixed with distilled water under heating, *p*H adjusted to 6·8, and the solution fed by a ball-tipped large hypodermic injection needle in doses of 200–1,000 mg./100 g. body wt. After that, the animals were permitted to subsist on stock diet and water, and kept under observation for 7 days or until death. Six rats were tested at each dose. A dose of 581·2 mg. per 100 g. body weight was found to be the Median Lethal Dose (LD_{50})[12].

Chronic toxicity dose: For studying chronic toxicity of ethyl gallate, growth, reproduction and general appearance of albino rats and lactation of their young ones were studied. Twenty weanlings from the stock-breeding colony were divided into 2 groups, each group containing 10 animals—5 male and 5 female. Each animal was kept in a separate cage. They were put on a diet containing: Crushed wheat, 75 ; baker's yeast powder, 4 ; casein, 8 ; cod liver oil, 2 ; Steenbock salt mixture, 3 ; and vanaspati (m.p. 37°C.), 8 per cent. The vanaspati in the diet of one group contained no ethyl gallate, and that of the other contained 1 per cent ethyl gallate. This large dose of ethyl gallate was selected purposely instead of a dose of 0·005 per cent or 0·01 per cent which has been used in vanaspati for storage experiments.

After 10 weeks on the experimental diet, the rats were paired for mating. After 3 to 4 weeks, when signs of pregnancy were apparent, the males were separated from the females. A record of weekly growth, young ones born, their number, average weight and length after birth and survival was maintained.

It was found by statistical analysis that there was no significant (at 5 per cent level) difference in the rate of growth of animals (males and females were separately compared) for the experimental period of 9 weeks on diet with and without ethyl gallate.

Regarding reproduction, comparable fertility was observed on both diets. The mean size and mean weight of the litters at birth were comparable irrespective of the diets. Thus, no difference in overall reproduction performance was observed in the animals fed with the two diets.

For examining lactation performance, the number of young born and of those which survived, total weight of the young after birth, total and average weight and length of the surviving young after 21 days, were recorded. In all these respects the performances of the two groups with and without ethyl gallate were comparable.

Liver function test: Robinson and co-workers[18] found that tannic acid (5–10 mg./kg.) injected intravenously produced considerable damage to the liver. Gallic acid being a degradation product of tannic acid might also produce damage. To investigate this point and also to extend the work on chronic toxicity, the liver function of rats taking ethyl

gallate was investigated. Unger *et al.*[19] showed that the ability to maintain a normal prothrombin level of blood is a function of the liver which is apt to be affected before other functions are sufficiently disturbed to permit clinical detection. They also claim that the response of the liver to parenteral administration of large doses of vitamin K in prothrombin production is a sensitive indicator of hepatic function.

One ml. of blood was taken by heart puncture from the two groups of rats (one receiving ethyl gallate and the other, no ethyl gallate) from the chronic toxicity experiments, with a 2 ml. syringe previously rinsed with 0.1 M sodium oxalate, and transferred to a cooled, similarly oxalate-treated conical centrifuge tube. 0.1 ml. of oxalated plasma was transferred to a pyrex tube (13 × 100 mm.) by means of a 0.2 ml. serological pipette. The prothrombin time was determined by the method of Quick[20].

After the normal prothrombin time was determined, each rat was injected daily for 2 days with a dose of 100 μg of synthetic vitamin K (Synkavit of *Hoffmann-La Roche*) in water and the prothrombin time was determined after 48 hr.

The 't' test was applied to the prothrombin time of both experimental and control animals, before and after injection. Before injection, there was no significant difference in the prothrombin time. Also, injection of vitamin K did not bring about any significant change in the test or in control animals, showing that ethyl gallate did not produce any liver dysfunction as indicated by the prothrombin test.

Acute toxicity dose of oxidised ethyl gallate: The possibility cannot be neglected that a toxic product might be formed when an antioxidant is oxidised in the course of its protective action on fats. Hence, oxidised ethyl gallate was tested for its acute oral toxicity.

Ethyl gallate was oxidised by blowing air at room temperature through an alkaline solution of ethyl gallate in water, until the test for ethyl gallate with sodium-potassium tartrate and ferrous sulphate[16] was negative. The solution was then neutralised and administered to experimental rats. Of the graded doses, the highest one used was 250 mg. of oxidised ethyl gallate per 100 g. body weight. This dose appeared to just exceed the limit of innocuousness. Though this was not absolutely harmless, none of the animals died and no serious toxic signs were apparent. Only, the urine was black for 3 to 4 days and the food consumption was less for the first two days of feeding after which it was normal again. On the other hand, 200 mg. of oxidised ethyl gallate per 100 g. body weight appeared to be innocuous; there was no blackening of the urine and no reduction in the food consumption.

Metabolism of ethyl gallate: In order to find out whether, after a long period of feeding of ethyl gallate in small concentrations, there was any accumulation of ethyl gallate, gallic acid and its derivatives, conjugate or residues in the tissues of the animals, the following procedure was adopted.

Animals from the chronic toxicity experiments were kept for 10 weeks on a diet containing ethyl gallate (1 per cent ethyl gallate in vanaspati which constituted 8 per cent of the diet). Taking the daily food consumption of each rat to be 10 g., the daily ethyl gallate intake

by each would be 8 mg. This amount or its fraction was chased in urine and feces (24 hr.), liver, brain, kidney and thigh muscle to see how ethyl gallate was disposed of in the body and if there was any cumulative storage in any of the tissues.

The absence of accumulation of ethyl gallate in tissues, its absence in feces, and presence (0.62 mg.) in urine indicated that ethyl gallate was metabolised quickly to smaller fragments or might have formed conjugates not detectable by the method used[16].

Summary

Straight-hardened vanaspati is more stable than blended vanaspati. The stability is roughly correlated with the linolein content of straight-hardened and blended vanaspati.

In respect of storage in closed tins, there is hardly any difference in peroxide value of vanaspati made from hydrogenated groundnut oil mixed with hydrogenated palm oil and that containing no palm oil; whereas, the acid value of vanaspati containing hydrogenated palm oil increases somewhat more rapidly than that of the other. In loose-cover tins, however, the peroxide values are lower in samples containing palm oil, though the acid values are greater. Vitamin A retention is slightly greater in samples containing hydrogenated palm oil in loose-cover tins than in samples containing no palm oil.

Among vanaspati samples prepared from hydrogenated groundnut oil and different proportions of hydrogenated linseed oil, the peroxide and acid values are higher in samples having greater proportions of hydrogenated linseed oil both in sealed tins and in loose-cover tins. This may be correlated with the higher iodine values of the samples. Samples with smaller proportions of hydrogenated linseed oil retained greater proportions of vitamin A.

Among the samples of vanaspati prepared from varying proportions of hydrogenated groundnut and mustard oils, the difference in respect of peroxide and acid values is not very marked in closed tins. In loose-cover tins, however, the peroxide and acid values are higher in samples containing greater proportions of hydrogenated mustard oil. Samples with smaller proportions of hydrogenated mustard oil preserve greater proportions of vitamin A.

Crude sesame oil stabilises vanaspati better than refined sesame oil or refined hydrogenated sesame oil. Besides, it gives greater colour intensity in the Baudouin test. The chromogenic property of vanaspati containing crude sesame oil also persists for longer periods of storage. Crude sesame oil does not appear to affect the colour, taste and smell of vanaspati when compared with refined and hydrogenated sesame oil.

Among the crude and refined groundnut oils and blended and straight-hardened vanaspati, straight-hardened vanaspati retains the maximum amount of vitamin A acetate both at 37.5°C. and 60°C.; refined oil retaining the least. Ethyl gallate affords a small but significant degree of protection to vitamin A acetate. Vitamin A acetate seems to promote instability of oils which, however, is counteracted to some extent by ethyl gallate. Rancidity (as indicated by odour) is not perceptibly promoted by vitamin A acetate.

Crude and hydrogenated sesame oils do not make any difference to the stability of vitamin A in vanaspati. With refined sesame oil, the stability of vitamin A is less. Ethyl gallate, as usual, is beneficial in all cases.

The rate of destruction, in storage at room temperature, of vitamin A acetate and Vanitin (*Hoffamann-La Roche*) used for enriching vanaspati is highest during the first fortnight, after which the rate gradually falls off. During storage in loose-cover tins, none of the antioxidants added, namely, ethyl gallate, propyl gallate, Tenox II and BHA improves the stability of vitamin A of Vanitin used for enriching vanaspati except NDGA which appears to exert a slightly protective action.

Storage in sealed tins at 37°C. causes marked loss of vitamin A of Vanitin and vitamin A acetate added to vanaspati prepared from hydrogenated groundnut oil at the end of the first month. Both BHA and ethyl gallate have some protective action under these conditions.

The longer the time and the higher the temperature of heating, the greater is the destruction of vitamin A in vanaspati containing vitamin A acetate. Some protection to vitamin A is afforded by ethyl gallate under these conditions also, as observed during the cooking of some preparations, viz., *halua*, potato chips and *puris*.

During heating of vanaspati enriched with Vanitin, loss of vitamin A is not very great at 110°C., but a loss of 50 to 100 per cent may occur at 250°C. Ethyl gallate affords some protection.

In the course of cooking food preparations using vanaspati enriched with Vanitin, a loss of vitamin A (from 15.8 to 33.4 per cent) has been observed. The loss may be considerable when the same oil is again used for frying. BHA offers some protection.

For the determination of physiological suitability of ethyl gallate as an antioxidant, the acute toxicity dose of ethyl gallate was determined. An oral dose of 581.2 mg. per 100 g. body weight of albino rats weighing 100 to 200 g. has been found to be the median lethal dose LD^{50}.

For chronic toxicity dose determination with ethyl gallate, growth, reproduction and lactation in rats receiving fairly high doses of ethyl gallate daily have been studied. No statistically significant difference is observed in the growth of rats maintained on diets with and without ethyl gallate. Reproduction and lactation are also unaffected by the presence of ethyl gallate in the diet.

Albino rats taking 1 per cent ethyl gallate in vanaspati, which constitutes 8 per cent of the diet, when tested for liver function do not show any difference from control rats.

In regard to the acute oral toxicity dose of oxidised ethyl gallate, a dose of 250 mg. of completely oxidised ethyl gallate per 100 g. body weight of rats appears to just exceed the limit of innocuousness. Though it is probably not completely harmless, none of the animals die and no serious toxic signs appear ; the colour of urine is, however, black for 3 to 4 days and food consumption is less for the first two days of feeding. Any dose lower than this is innocuous.

Ethyl gallate is metabolised quickly to smaller fragments in rats. This is evident from the results, as it does not accumulate in tissues and is absent from the feces ; it is present in small amounts in the urine.

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II

Isolation and Identification of Iso-Oleic Acids

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Studies on the toxicity of nickel present in vanaspati and the nutritive value of iso-oleic acids[1] in hydrogenated oils have revealed that the small amount of nickel does not produce any toxic symptoms in monkeys and that the iso-oleic acids have almost the same nutritive value as the nutritive value of oleic acid. After it had thus been unequivocally proved that vanaspati is not harmful for human consumption, attention was directed towards the isolation and identification of the iso-oleic acids of vanaspati and some natural fats. In the present investigation, the isolation of iso-oleic acids using the urea adduct method has been accomplished. The details regarding the isolation procedure of iso-oleic acids of vanaspati and beef body fat are described. The identification of iso-oleic acids of vanaspati and cow butter fat has been carried out using paper chromatography. For the quantitative estimation of these acids, a method involving partition chromatography using silica gel has been developed. Using this method, the composition of the iso-oleic acid fraction of vanaspati, beef body fat and cow butter fat has been estimated and the results are presented below.

1. METHODS OF ISOLATION

Several reports have appeared from time to time to show the presence of isomerides of oleic acid in natural fats. Although many of the earlier claims have not survived the scrutiny of modern investigation, the identity of a few naturally occurring iso-oleic acids has been conclusively established. Petroselinic acid accompanies oleic and linoleic acids as a major component in the seed fats of the family *Umbelliferae*[2]. *Trans*-octadec-11-enoic acid was observed by Bertram[3] in small quantities in butter fat and the body fats of sheep and oxen. The presence of *trans* unsaturated monoethenoid acids in several ruminant and non-ruminant fats has been recently reported[4]. Apart from the naturally occurrring ones, a number of iso-oleic acids have been obtained by various chemical reactions from natural oleic acid or acids related to it. The most important reaction in this category is the catalytic hydrogenation of oils. Hydrogenation involves

mainly the conversion of unsaturated esters to partially or fully saturated ones. When the process of hydrogenation of oils is arrested before the unsaturated fat has been completely saturated with hydrogen, as is done with a great portion of the fat which is hydrogenated commercially for the production of vanaspati, certain complications become evident in the reaction. During hydrogenation, apart from the production of saturated acids by simple addition of hydrogen at the double bonds, isomerisation of some of the unsaturated fatty radicals also takes place. The so-called iso-oleic acids are the unsaturated acids which precipitate with the saturated acid fraction in the Twitchell lead salt-alcohol separation.

The most commonly employed method for obtaining a fraction rich in iso-oleic acids from fats, such as vanaspati or beef body fat, is to subject the mixed fatty acids (M.F.A.) to the procedure of Twitchell[5] for the separation of 'solid' and 'liquid' acids followed by Bertram[3] separation of solid saturated acids from solid *trans* unsaturated acids. The former is based on the differences in the solubilities of lead salts in ethanol, whereas the latter is based on the differences in the same property of mercuric salts in a mixture of methanol and glacial acetic acid. Both these methods are slow, costly and not particularly efficient. Though the modification suggested by Cocks, Christian and Harding[6] effects a greater recovery of the *trans* acids than the original Twitchell process, it is still not quantitative, especially if the *trans* acids are present in small quantities. Belekar and co-workers[8] cite in addition to their own results several instances of earlier work which report discrepancies in the saturated acid content of fats as determined by calculation from the iodine and thiocyanogen values and by the lead salt separation method. Low temperature crystallization from suitable solvents and fractional distillation of methyl esters have not found much favour for the segregation of the iso-oleic acid fraction from fats. For example, Belekar *et al.*[8] found that low temperature crystallization of hydrogenated fats from solvents such as acetone leads to the even distribution of iso-oleic acids in all the fractions. Magar[9] using the ester fractionation method for determining the component acids of vanaspati, could not isolate a fraction rich in iso-oleic acids.

The urea adducts of fatty acids have been investigated extensively for the segregation of the component acids of natural fats ; the various factors governing the selectivity of the formation of adducts of particular components from a mixture of fatty acids have been studied and well summarized by Ananth Narayan and Kulkarni[10]. Among unsaturated compounds, conjugation and *trans* configuration facilitate adduct formation compared with non-conjugation and *cis* configuration.

Mehta and co-workers[11] have applied the urea adduct technique, for the first time, for the fractionation of the component acids of hydrogenated fat. By the stepwise addition of urea to a solution of fatty acids in ethanol, they effected a separation of several fractions of progressively increasing iodine values ; however, fractions rich in iso-oleic acids have not been indicated by them. For obtaining the iso-oleic acid fraction, they first subjected the mixed fatty acids to a lead salt separation and then subjected the 'solid' acids to fractionation by the urea adduct method. In our earlier investigations on the composition of iso-oleic acids of fats, the iso-oleic acids were obtained by the method of Bertram[3]. In the present investigation, the urea adduct technique has been compared with the usual method for the separation of fatty acids of hydrogenated fat and beef body fat.

Methanol was employed as the solvent and a process of fractional crystallization of the adducts was chosen in preference either to the stepwise addition of urea to the solution of the fatty acids or to repeatedly subjecting the different acid fractions to treatment with urea. The stability of urea adducts in methanol solution even in the presence of a small amount of water, suggested that repeated crystallization of adducts themselves may prove useful thereby avoiding the necessity of liberating the acids in the intermediate stages and recombining them with urea under varying conditions of concentration and molar ratio of acids and urea.

Experimental

Materials

The methanol used in these experiments was of the best commercially available quality and was distilled before use. Urea (Merck), dried in vacuum at 45° for 4 hr., was used in saturated solution in methanol. The sample of vanaspati was the 'Dalda' brand manufactured by *Hindustan Vanaspati Manufacturing Co. Ltd.*, Bombay, obtained from the local bazaar; the mixed fatty acids had an iodine value, 71.0 and neutralization equivalent, 288.9. The beef body fat was obtained by heat-rendering of subcutaneous tissue from the abdominal portion of ox collected from the local slaughter house ; the mixed fatty acids had iodine value, 36.4 and neutralization equivalent, 277.0.

Procedure

The procedures adopted for the isolation of iso-oleic acids from vanaspati and beef body fat are summarized in Charts 1 and 2 respectively. The mixed fatty acids (1 part) were added to a hot solution of urea (4 parts) in methanol (25 parts w/v), refluxed for a few minutes and allowed to stand for 12 hr. at room temperature (18°–20°). The crystalline adducts separating out were filtered under slight suction without solvent washing, freed from solvent under vacuum in a desiccator and their weights determined. The raffinate was concentrated by evaporating off a specified portion of the solvent and the concentrated solution allowed to stand for 12 hr. at room temperature when a further crop of the adducts separated, which was isolated as before. The filtrate was reserved for the liberation of the acids.

Further treatment given to the adducts is shown in Charts 1 and 2. In all cases the adducts were crystallized after dissolution by refluxing with the specified amounts of methanol, which were arrived at from preliminary runs in which the iodine values of the separated fractions were determined. The filtrates were freed of methanol and the residues obtained were either recrystallized or admixed with the appropriate fractions. The crystallized adducts or raffinates were hydrolysed with slightly acidulated water, the liberated acids extracted with ether, the extracts washed with water, dried over anhydrous magnesium sulphate and the fatty acids isolated.

The acid fractions A and B in Chart 1 were pooled together and subjected to mercury salt separation. The acids (17 g.) were melted and added hot to a solution of mercuric acetate (17 g.) in methanol (17 ml.) and glacial acetic acid (13 ml.). After allowing to stand for 12 hr. the solids that separated were filtered and pressed (no washing). The filtrate

was decomposed with 15 ml. of concentrated hydrochloric acid with ice cooling, and the unsaturated acids in mixture with their methyl esters were taken up in ether. The ether solution was washed with hydrochloric acid and water, dried and ether removed. The methyl esters were saponified, and the acids were liberated and isolated (7.66 g.; iod. val. 80.5). In the case of beef body fat, the acids (12.7 g.) from A_1 (Chart 2) were subjected to mercury salt separation to give 1.5 g. of solid unsaturated acids (iodine value, 72.8).

Results and Discussion

The procedure evolved tends to isolate the saturated acids at one end and the unsaturated liquid acids at the other. The solubilities of the urea adducts of fatty acids resemble those of the lead salts ; the adducts of the 'solid' acids, both saturated and unsaturated, are less soluble in methanol than those of the liquid ones. Among the solid acids, the adducts of *trans* unsaturated acids are more soluble than those of saturated ones which are the least soluble in methanol. The recrystallization steps employed in the case of vanaspati and beef body fat differ only in the volume of methanol used, larger volumes of the solvent being necessary to separate iso-oleic acids from the saturated acid fractions in the case of vanaspati, which is richer in solid unsaturated acids than beef body fat. Also, since the liquid acid fraction is more in beef body fat than in vanaspati, larger volumes of the solvent are required to free the adducts from liquid acids.

The solid acid fractions A and B (Chart 1), iodine values, 52.6 (m.p., 44°–47°) and 61.5 (m.p., 31°–34°) respectively represented 35 per cent of the total mixed fatty acids. The corresponding fraction A_1 (Chart 2) from beef body fat had an iodine value of 16.2 and amounted to 25.8 per cent of the total acids. These figures compare somewhat favourably with the yields of 48.4 and 46.8 per cent of the 'solid' acids with iodine values 49.8 and 5.7 respectively, obtained from the mixed fatty acids of these two fats by Twitchell's procedure. Since a large number of crystallization steps would be necessary to obtain a fraction composed mostly of solid unsaturated acids, the final enrichment of fractions A and B from vanaspati and A_1 from beef body fat was effected by the method of Bertram. The iso-oleic acid fractions thus obtained in overall yields of 17.0 per cent from vanaspati and 3.0 per cent from beef body fat had iodine values of 81.2 and 72.8 respectively. The iso-oleic acid fractions obtained through a combination of the methods of Twitchell and Bertram in yields of 19.7 per cent from vanaspati and 0.9 per cent from beef body fat had iodine values of 88.5 and 77.2 respectively. The yields of the final fractions of comparable iodine value obtained by the present method are better in the case of beef body fat and are of the same order in the case of vanaspati in comparison with their yields through a combination of the methods of Twitchell and Bertram.

Since the final fractions obtained are solid at room temperature and have high iodine values, they are probably rich in *trans* acids. For accurate estimations of the proportions of *cis* and *trans* isomers, infra-red studies are necessary and they are being undertaken.

2. IDENTIFICATION OF ISO-OLEIC ACIDS

Vanaspati

Hilditch and Vidyarthi[12] found that hydrogenation of methyl oleate

CHART 1—Isolation of Iso-oleic Acid Fraction from Mixed Fatty Acids of Vanaspati

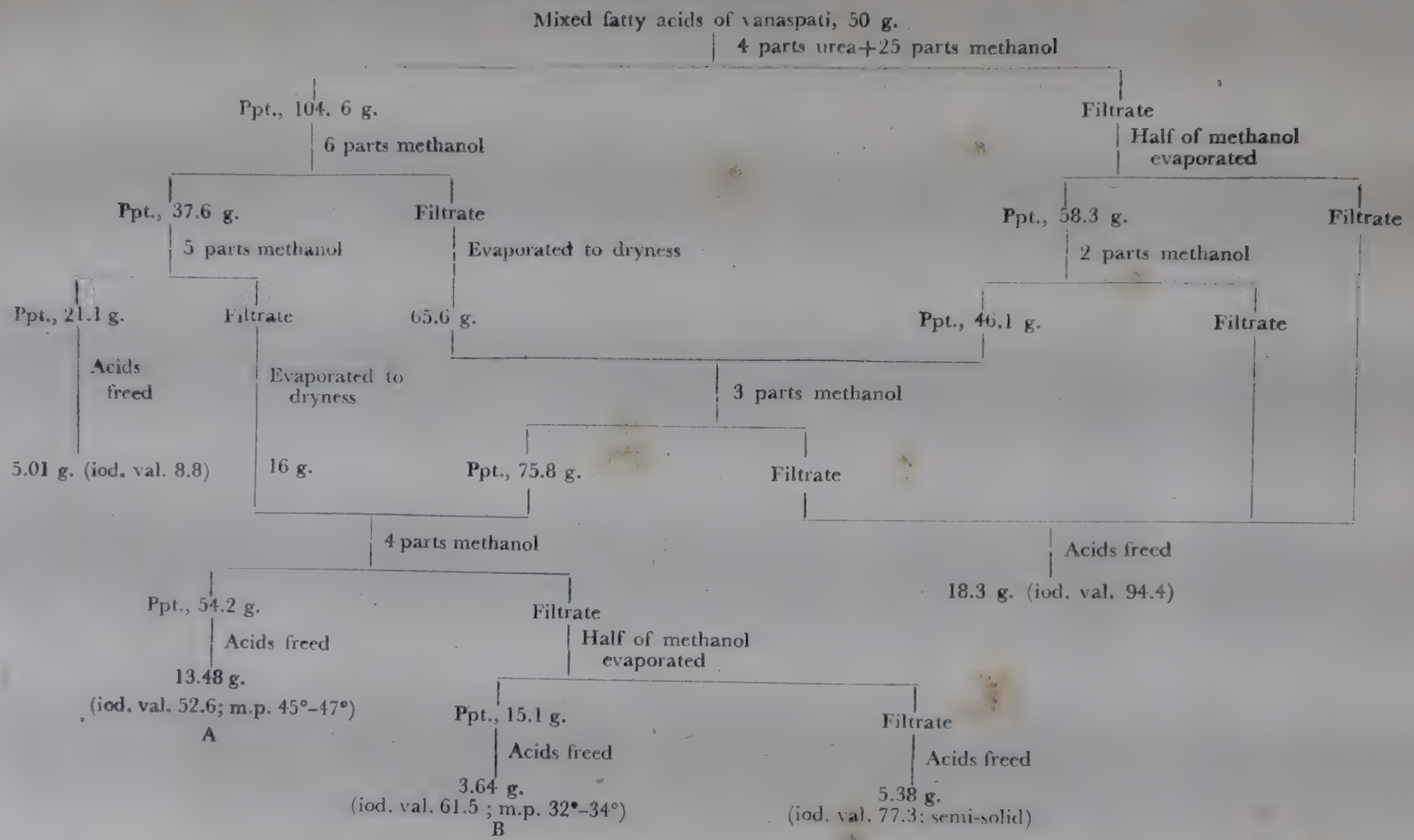
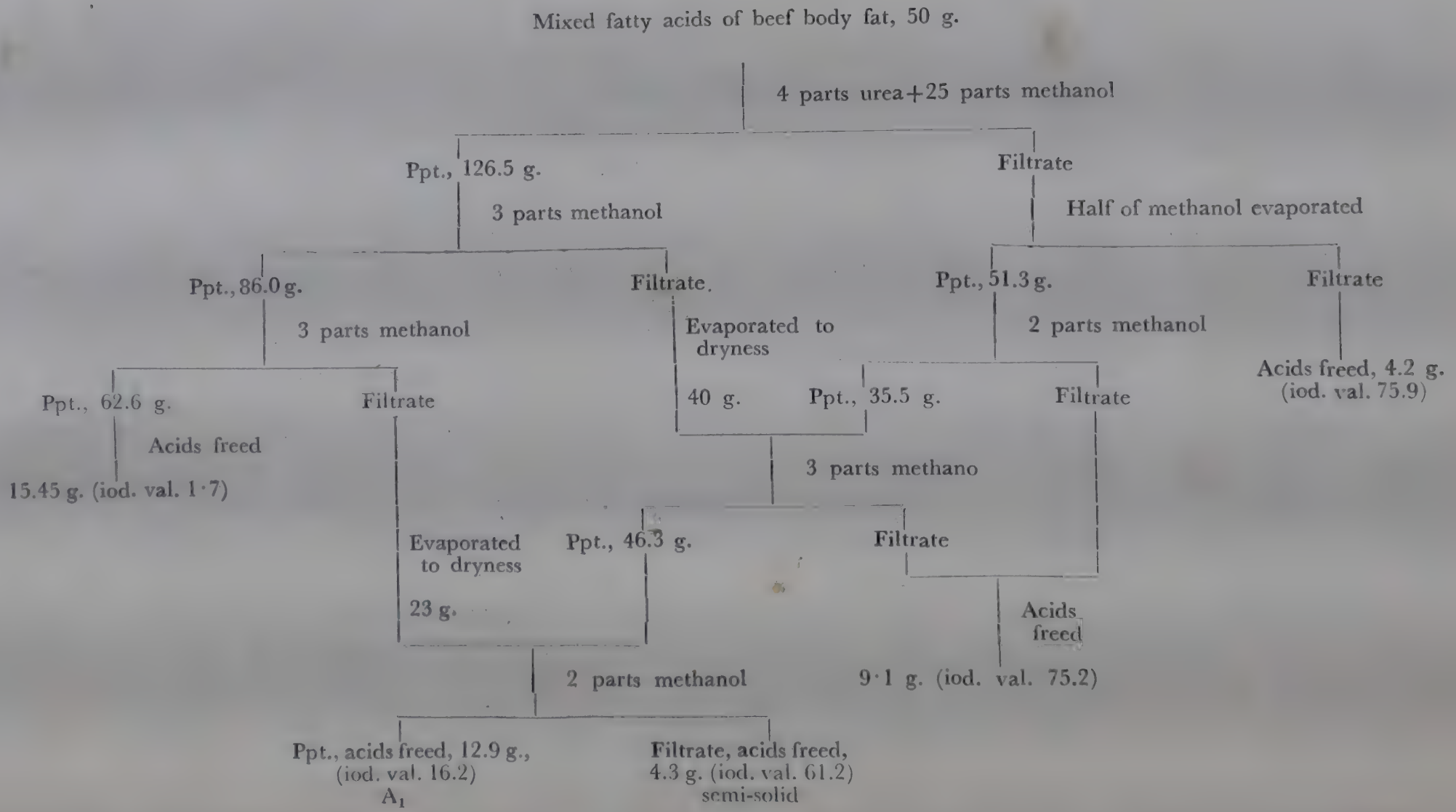


CHART 2—Isolation of Iso-Oleic Acid Fraction from Mixed Fatty Acids of Beef Body Fat



when carried out to reduce the iodine value by about 30 per cent resulted in the formation of both positional and geometrical isomers. They found that, in addition to elaidic acid, both $\triangle^{-6}$ and $\triangle^{-10}$ octa-decenoic acids are produced during the partial hydrogenation process. No study has so far been reported on the nature of individual isomers present in the solid iso-oleic fraction of vanaspati and hence the present study was undertaken. The identification of isomers of oleic acid in a mixture by separating the acids themselves is difficult to accomplish. As an alternative, the acids are oxidised and the oxidation products are identified by suitable methods. Among the most commonly employed methods for the oxidation, the one due to Armstrong and Hilditch[18] modified by Begemann, Keppler and Boekenoogen[14] appears to give almost quantitative results. In this method, the fatty acids are oxidised by potassium permanganate in acetic acid. As a result of this oxidation, a mixture of mono- and dicarboxylic acids results :

$$CH_3(CH_2)CH:CH(CH_2)COOH \longrightarrow CH_3\ (CH_2)\ COOH + HOOC(CH_2)COOH$$

The position of the double bond in the starting material can be deduced by the identification of the resulting acids. Of the monocarboxylic and the dicarboxylic acids, the former seem to be subject to secondary decomposition, while the latter are stable. The identification of the dicarboxylic acids is, therefore, preferred for establishing the presence of individual components of a mixture of monoethenoid acids. For the separation of the components of a mixture of dicarboxylic acids, the classical methods of fractional crystallisation and distillation[12] are too cumbersome. In the paper chromatographic method of Reid and Lederer,[15] small quantities of the acid mixture can be used and therefore this method was chosen for the separation of the mixture of dicarboxylic acids obtained on oxidation of iso-oleic acids of vanaspati.

Whatman No. 1 filter paper was cut into squares (12 in. side) and kept in an atmosphere of ammonia for 4 hr. before use. The acid mixture was spotted as its ammonium salts (120 μg. in 20 μl.), side by side with a mixture of synthetic acids. The butanol phase of a well shaken mixture of equal volumes of butanol and ammonia (1.5 per cent) served as the developing solvent. After developing for 40 hr., the paper was removed, dried in a current of air and sprayed with a solution of bromocresol purple in a mixture of ethyl alcohol and formaldehyde (5 : 1). The paper was then dried and exposed to an atmosphere of ammonia for 3 min. The acids showed themselves as yellow spots on a purple background. By this procedure, acids from suberic to dodecanedioic acids could be separated on paper and the Rf values of the acids were as follows : suberic 0.085 ; azelaic, 0.14 ; sebacic, 0.20 ; undecanedioic, 0.31 ; and dodecanedioic acid, 0.40. Fig. 1 represents the separation of synthetic acids spotted individually and in mixture. It can be seen from the figure that the synthetic acids are separated into five spots. The Rf values of the acids in the mixture corresponded to those obtained when the acids were spotted individually. Fig. 2a shows the separation of a mixture of suberic, azelaic, sebacic, undecanedioic and dodecanedioic acids and Fig. 2b shows the separation of dicarboxylic acids produced on oxidation

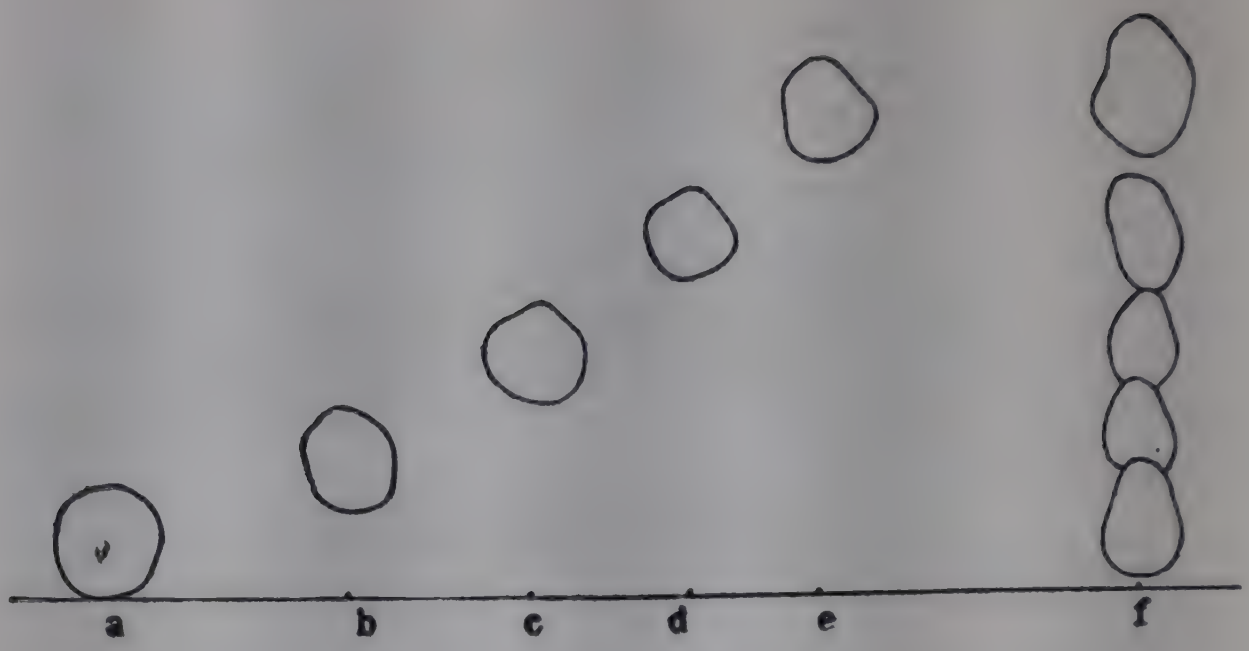


FIG. 1—PAPER CHROMATOGRAPHY OF INDIVIDUAL ACIDS SPOTTED SEPARATELY (a–e) AND MIXTURE OF SUBERIC, AZELAIC, SEBACIC, UNDECANEDIOIC AND DODECANEDIOIC ACIDS (f)

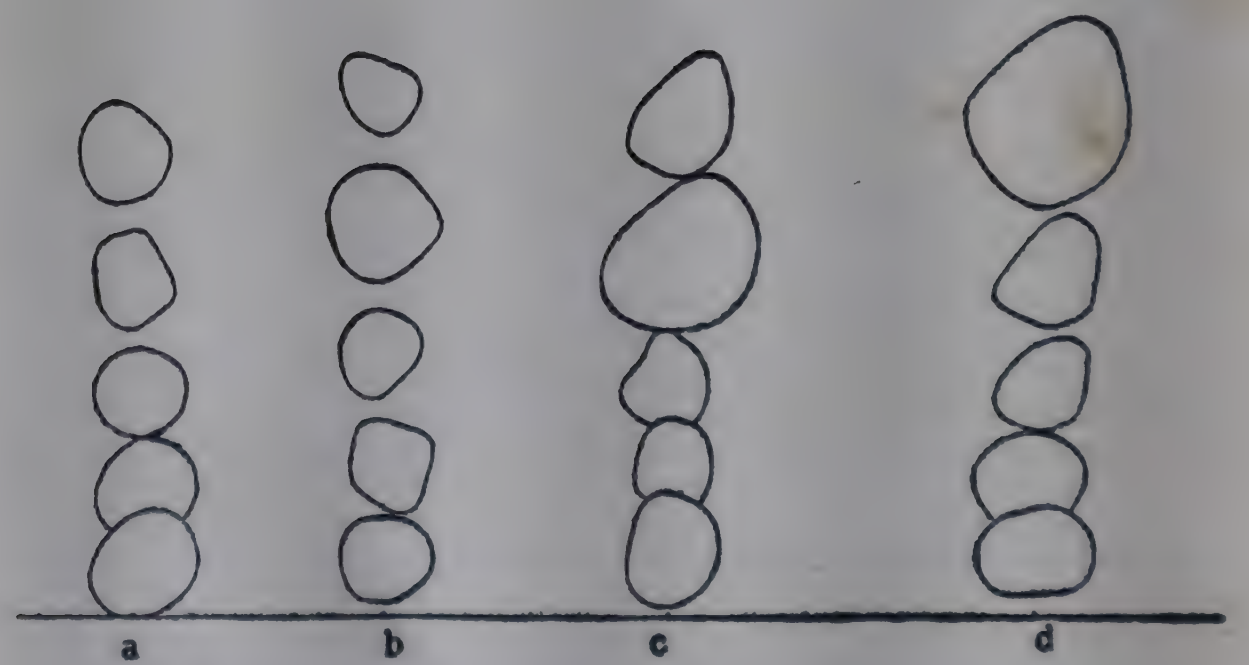


FIG. 2—PAPER CHROMATOGRAPHY OF (a) SYNTHETIC ACID MIXTURE, (b) DIBASIC ACIDS OF VANASPATI, (c) VANASPATI PLUS C_{11} AND (d) VANASPATI PLUS C_{13}

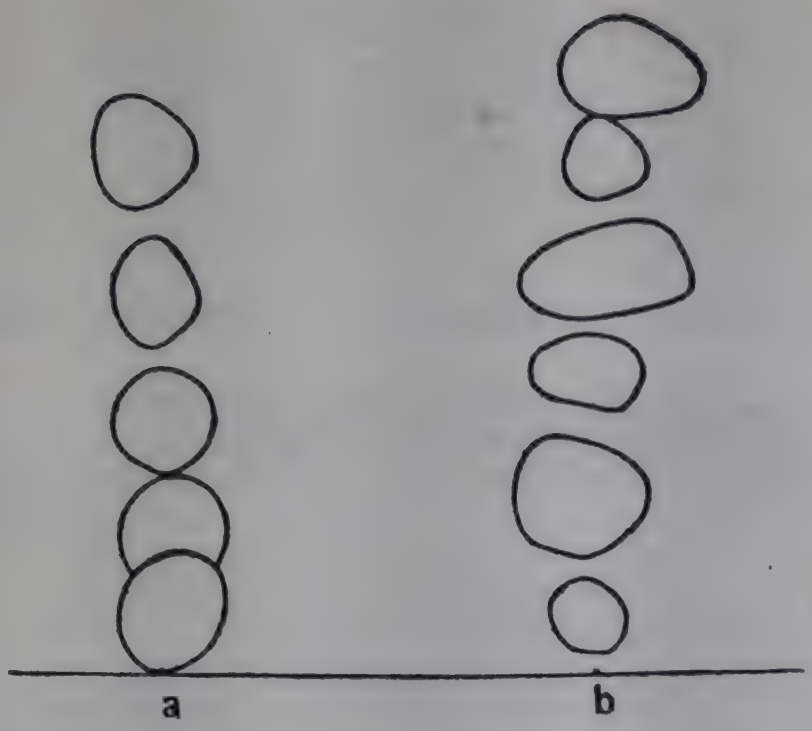


FIG. 3—PAPER CHROMATOGRAPHY OF (a) SYNTHETIC ACID MIXTURE AND (b) DIBASIC ACIDS OF COW BUTTER FAT

of iso-oleic acids of vanaspati. This shows that dicarboxylic acids from suberic to dodecanedioic acids are present in the dicarboxylic acid mixture, thereby indicating the presence of $\triangle^{-8}$, $\triangle^{-9}$, $\triangle^{-10}$, $\triangle^{-11}$ and $\triangle^{-12}$ octadecenoic acids in the hydrogenated fat sample. Figs. 2c and 2d show the separation of dicarboxylic acid mixture from vanaspati with added undecanedioic and dodecanedioic acids respectively. Here also, the acids are separated into five fairly well defined spots ; the spot containing the C_{11} acid intensified the fourth spot from the starting line and the one containing C_{12} intensified the fifth spot. Thus, using the intensification technique, the positions of the dicarboxylic acids were confirmed.

Cow Butter Fat

Vanaspati is often used as an adulterant of ghee (clarified butter). As the only C_{18} monoethenoid acids present in butter so far known are oleic and vaccenic acids, it was thought that it should be possible to detect adulteration with hydrogenated oil by paper chromatography of the dicarboxylic acids obtained on oxidation of the adulterated product. As a preliminary step, it was decided to start with ghee prepared from pure cow butter fat. Accordingly, the solid fatty acid fraction obtained from cow butter fat was subjected to mercury salt precipitation following the procedure of Bertram[3]. The solid unsaturated acids thus obtained were converted into their methyl esters and oxidised in the same manner as was done for vanaspati. Dicarboxylic acids that were formed were subjected to paper chromatography as described before. Fig. 3b illustrates the separation of the dicarboxylic acid mixture. It can be seen from the figure that the dicarboxylic acids are separated into 6 different zones. Fig. 3a shows the separation of the mixture of synthetic acids. Thus the chromatographic identification of the five dicarboxylic acids leads to the conclusion

that, in addition to oleic acid, other octadecenoic acids with double bonds at $\triangle^{-8}$, $\triangle^{-10}$, $\triangle^{-11}$ and $\triangle^{-12}$ are present in cow butter fat. The sixth spot in Fig. 3b is probably due to tridecanedioic acid which in turn might have arisen by the oxidation of $\triangle^{-13}$ octadecenoic acid. This observation requires further confirmation.

3. QUANTITATIVE ESTIMATION OF ISO-OLEIC ACIDS

The chromatographic method, while establishing the presence of various octadecenoic acids in butter fat and vanaspati, is not helpful in the quantitative estimation of iso-oleic acids. Moreover, the lack of sharpness of the outline of spots and their transient nature preclude even a rough estimation of the quantitative composition of the mixture. In order to separate the dicarboxylic acids individually and to identify them by the preparation of suitable derivatives, it is necessary to use materials in amounts larger than are used normally for paper chromatography. Hence the desirability of a chromatographic separation of dicarboxylic acids on a column became evident. Another point to be considered in the quantitative estimation of dicarboxylic acids is the disruptive oxidation of iso-oleic acids. Begemann *et al.*[14] suggested some modification in the disruptive oxidation technique and used acetic acid as solvent instead of acetone. On oxidation using acetic acid, nearly 90 per cent of the dicarboxylic acids can be recovered by analysis. It is desirable to effect the separation of dicarboxylic acids even in the presence, in the mixture, of small amounts of monocarboxylic acids. For, in the analysis of oxidation products of iso-oleic acids, the method employed[14] to separate monocarboxylic acids from dicarboxylic acids is to extract the oxidation products repeatedly with hot petroleum ether. Such a leaching out procedure is apt to remove some of the dicarboxylic acids as well and the proportionate loss of minor constituents of the mixture may often be quite significant. If the dicarboxylic acids could be separated and estimated even in the presence of small amounts of monocarboxylic acids, the number of extractions of the oxidised mixture with petroleum ether could be minimised and the results obtained would represent a truer picture of the original composition of the dicarboxylic acids and consequently of the iso-oleic acid fraction.

Preliminary experiments indicated that buffered columns used by Klenk and Bongard[16], Vandenheuvel and Hayes[17], and Higuchi, Hill and Corcoran[18] were not effective in the facile separation of a mixture of dicarboxylic acids ranging from suberic to dodecanedioic acids. The method employed by Begemann *et al.*[14] for the elucidation of the structures of monoethenoid acids effects a quantitative separation of dicarboxylic acids, but suffers from the disadvantage that large quantities of the mixture cannot be separated. A method which allows the separation of a mixture of acids up to 100 mg. has been developed in the present investigation.

Outline of the method: Silica gel serves as the inert support in the present method of partition chromatography of the acids. The aqueous layer of a well shaken mixture of 3 parts by volume of ethanol, 4 parts methanol, and 3 parts water to 10 parts benzene serves as the stationary phase while the benzene layer of the same mixture is utilized as the eluting phase. After the acids begin to get eluted, the eluate is collected in fractions of suitable volumes and titrated against standard alkali. A plot of the

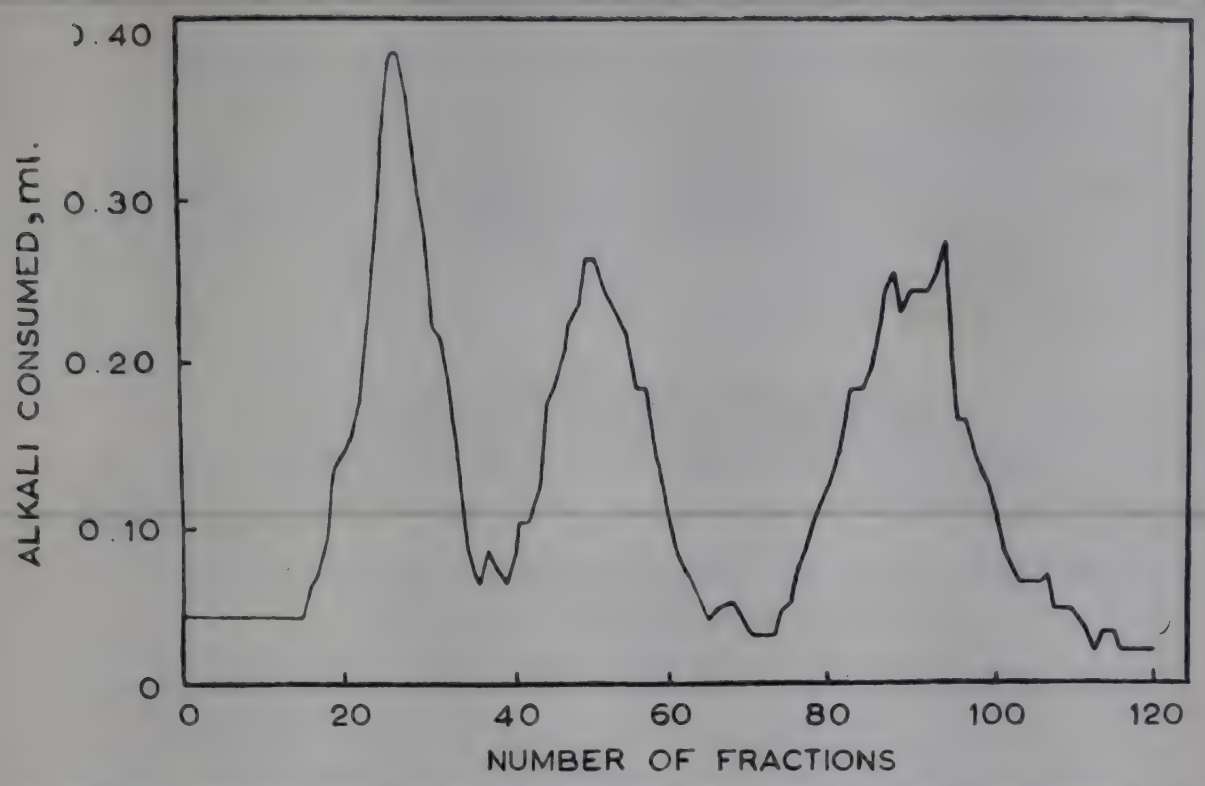


FIG. 4—CHROMATOGRAPHIC RUN OF A MIXTURE OF SEBACIC, AZELAIC AND SUBERIC ACIDS IN KNOWN PROPORTIONS

volume of alkali consumed against the number of fractions shows that the acids are eluted at well defined intervals and in the decreasing order of their C-chain lengths. It is apparent that the volume of alkali needed to neutralise each of the bands is also a quantitative measure of the acid comprising it. Further, concentration of eluent fractions which have been thus neutralised yields a solution of the sodium salt of the respective acids from which the acid itself can be liberated by acidification. The solution can be directly used for the preparation of derivatives by treatment with reagents such as *p*-bromophenacyl bromide.

Separation of the components of synthetic acid mixtures: With a view to standardise the method for the separation of dicarboxylic acids, a mixture comprising known amounts of sebacic, azelaic and suberic acids was submitted to chromatography. The results given in Table 1 and Fig. 4 show that the components of the mixture were clearly separated in three well defined peaks. The recoveries of acids (based on the volume of alkali consumed by the respective peaks) were quite satisfactory. It may be added that consistent recoveries were obtained in repeated runs. The order of elution of acids and their identities were confirmed by the isolation of acids from the corresponding eluates from the column. Thus, the fractions comprising a single peak were pooled together and the solution was concentrated to a small volume (*c.* 5–10 ml.) by evaporation on a water bath. A portion of the concentrate was acidified with hydrochloric acid (1 N) and the acids precipitated were filtered off and repeatedly crystallised from hot water. The acids isolated from the synthetic acid mixture had the following melting points: sebacic, 133–134° (literature, 134°); azelaic, 107–108° (literature, 108°); and suberic, 139–140° (literature, 140°). A further portion of the solution of sodium salt was treated with *p*-bromophenacyl bromide in the conventional manner. The derivatives of sebacic and azelaic acids thus obtained had the following melting points: sebacic

Table 1—Separation of Acids from Synthetic Acid Mixture

(*Length of column, 53 cm. ; wt. of silica gel, 15 g. ; wt. of mixture. 30.6 mg.*)

Acid	Fraction	Alkali (0.030N) consumed *ml.*	Recovery	
			mg.	%
Sebacic	18–40	3.68	11.52	102.9
Azelaic	42–77	3.22	8.92	103.7
Suberic	78–117	4.23	10.85	100.5

Table 2—Separation of Acids from a Mixture of Dicarboxylic Acids with added Palmitic Acid

(*Length of column, 39.8 cm. ; wt. of silica gel, 55 g. ; vol. of neutral eluate, 15 ml.*)

Acid	Fraction	Alkali (0.033N) consumed *ml.*	Recovery	
			mg.	%
Palmitic	7–40	2.94	24.79	116.6
Sebacic	82–141	8.10	26.94	91.0
Azelaic	142–167	10.93	33.96	100.7
Suberic	168–203	11.63	33.20	99.7

acid derivative, 145–146° (literature, 147°) ; and azelaic acid derivative, 130–131° (literature, 131°).

The results of attempts to separate the dicarboxylic acids in the presence of monocarboxylic acids have proved to be of great value. Fig. 5 (Table 2) depicts the results obtained with a mixture of known composition. The mixture was made up of known amounts of sebacic, azelaic and suberic acids with commercial grade palmitic acid. It will be seen from Fig. 5 that the monocarboxylic acids are eluted ahead of the dicarboxylic acids in a single peak. Experiments with a mixture of sebacic, azelaic, suberic, palmitic, stearic and lauric acids support this observation. The rather high recoveries in the case of monocarboxylic acids may perhaps find an explanation in the presence of certain unspecified impurities in commercial samples. However, the results show conclusively that for the assay of dicarboxylic acids, complete removal of monocarboxylic acids from the mixture is not essential.

As stated earlier, the dicarboxylic acids are formed in conjunction with monocarboxylic acids on the oxidation of iso-oleic acids. As the present method of assay enables a quantitative determination of dicarboxylic acids to be made even in the presence of small amounts of monocarboxylic acids, it is well adapted for the quantitative study of the iso-oleic acid fractions of fats.

Iso-oleic acids of vanaspati: Solid iso-oleic acids are found in vanaspati to the extent of 25–30 per cent. The iso-oleic acid fraction has been separated and the individual components have been qualitatively identified by paper chromatography.

For the quantitative estimation of the acids, the iso-oleic acid fraction was isolated from a sample of vanaspati according to the procedure of Bertram[3] and then oxidised in the form of their methyl esters. The mixture of dicarboxylic acids was isolated from the oxidation products according to the procedure of Begemann *et al.*[14] The results obtained from a representative run are :

	Weight g.	Iod. val.
Vanaspati	100.0	65.6
Mixed fatty acids	91.0	69.0
Solid fatty acids	44.0	49.8
Iso-oleic acids	19.7	88.5
Methyl esters of iso-oleic acids	18.5	83.0
Dicarboxylic acids from 5.01 g. of the ester	3.01	..

Fig. 6 (Table 3) represents the results obtained with a sample of dicarboxylic acids from vanaspati and Fig. 7 (Table 4) gives the results obtained with the sample after adding a known amount of suberic acid. It will be seen that the mixture of dicarboxylic acids is cleanly separated into five acids, ranging from suberic to dodecanedioic acids, confirming the observation made earlier using paper chromatographic methods. The quantitative composition of the mixture is given in Table 5.

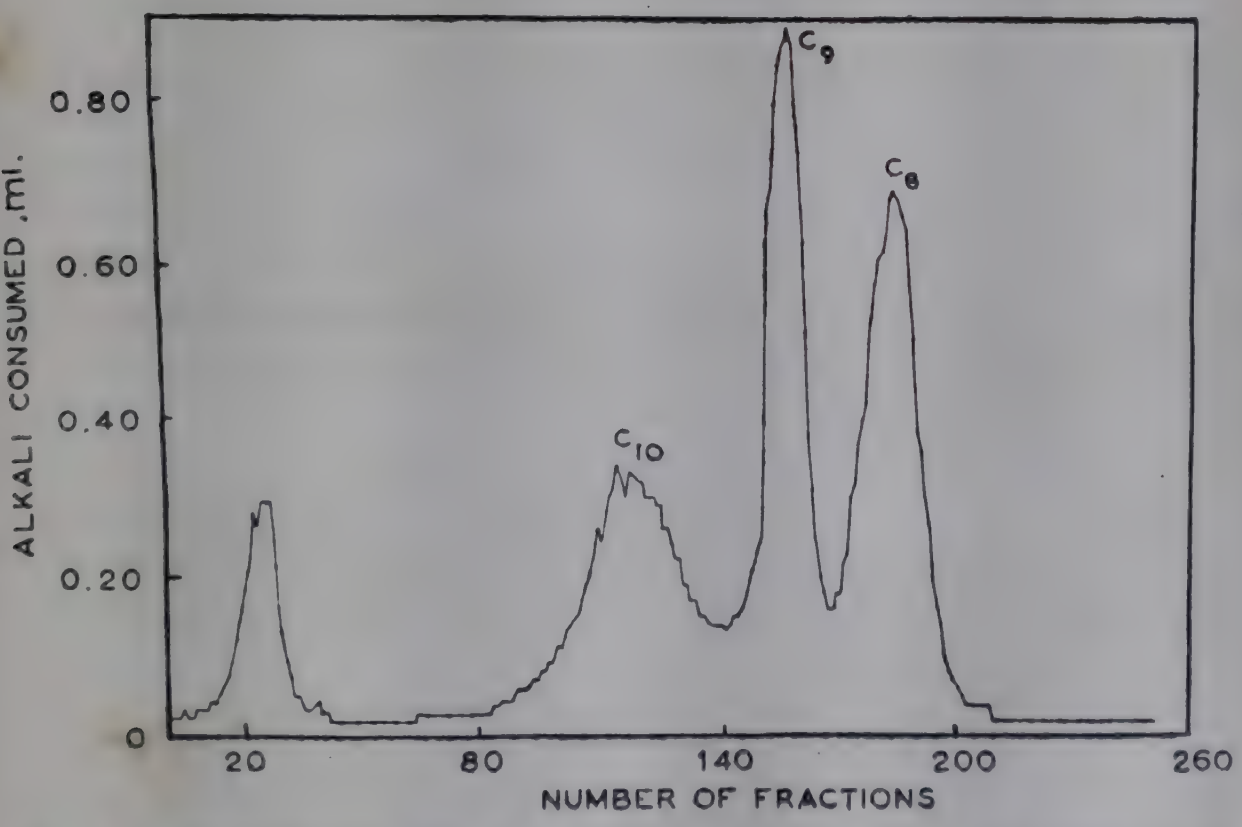


FIG. 5—CHROMATOGRAPHIC RUN OF A MIXTURE OF SEBACIC, AZELAIC AND SUBERIC ACIDS WITH ADDED PALMITIC ACID IN KNOWN PROPORTIONS

TABLE 3—DICARBOXYLIC ACIDS IN OXIDATION PRODUCTS OF VANASPATI ISO-OLEIC ACIDS

(*Length of column, 52 cm. ; wt. of silica gel. 43.0 g. ; wt. of mixture, 85.0 mg. ; recovery, 94.7% ; vol. of fractions: 1–100, 1 ml. ; 161–295, 3 ml.*)

Acid	Fraction	Alkail (0.033N) consumed *ml.*	Composition *mg.*
Dodecanedioic	10–54	1.11	4.26
Undecanedioic	56–103	1.55	5.58
Sebacic	114–174	4.24	15.22
Azelaic	175–231	13.35	46.46
Suberic	232–288	1.95	8.98

TABLE 4—DICARBOXYLIC ACIDS IN OXIDATION PRODUCTS OF VANASPATI ISO-OLEIC ACIDS WITH ADDED SUBERIC ACID

(*Length of column, 53 cm. ; wt. of silica gel, 41 g. ; wt. of mixture, 74.4 mg. ; wt. of suberic acid added, 8.0 mg. ; recovery, 104.0% ; vol. of fractions: 1–150, 1 ml. ; 151–250, 3 ml.*)

Acid	Fraction	Alkali (0.034N) consumed *ml.*	Composition *mg.*
Dodecanedioic	8–48	1.00	4.43
Undecanedioic	49–91	1.38	5.74
Sebacic	97–141	3.69	14.35
Azelaic	151–187	12.15	43.97
Suberic	195–232	5.13	17.20

TABLE 5—PERCENTAGE COMPOSITION OF MIXTURE OF DICARBOXYLIC ACIDS FROM VANASPATI

(*Calculated from Figs. 6 and 7*)

Acid	From Fig. 6	From Fig. 7
	%	%
Dodecanedioic	5.7	5.3
Undecanedioic	7.4	6.9
Sebacic	18.6	18.9
Azelaic	56.8	57.7
Suberic	11.5	11.2

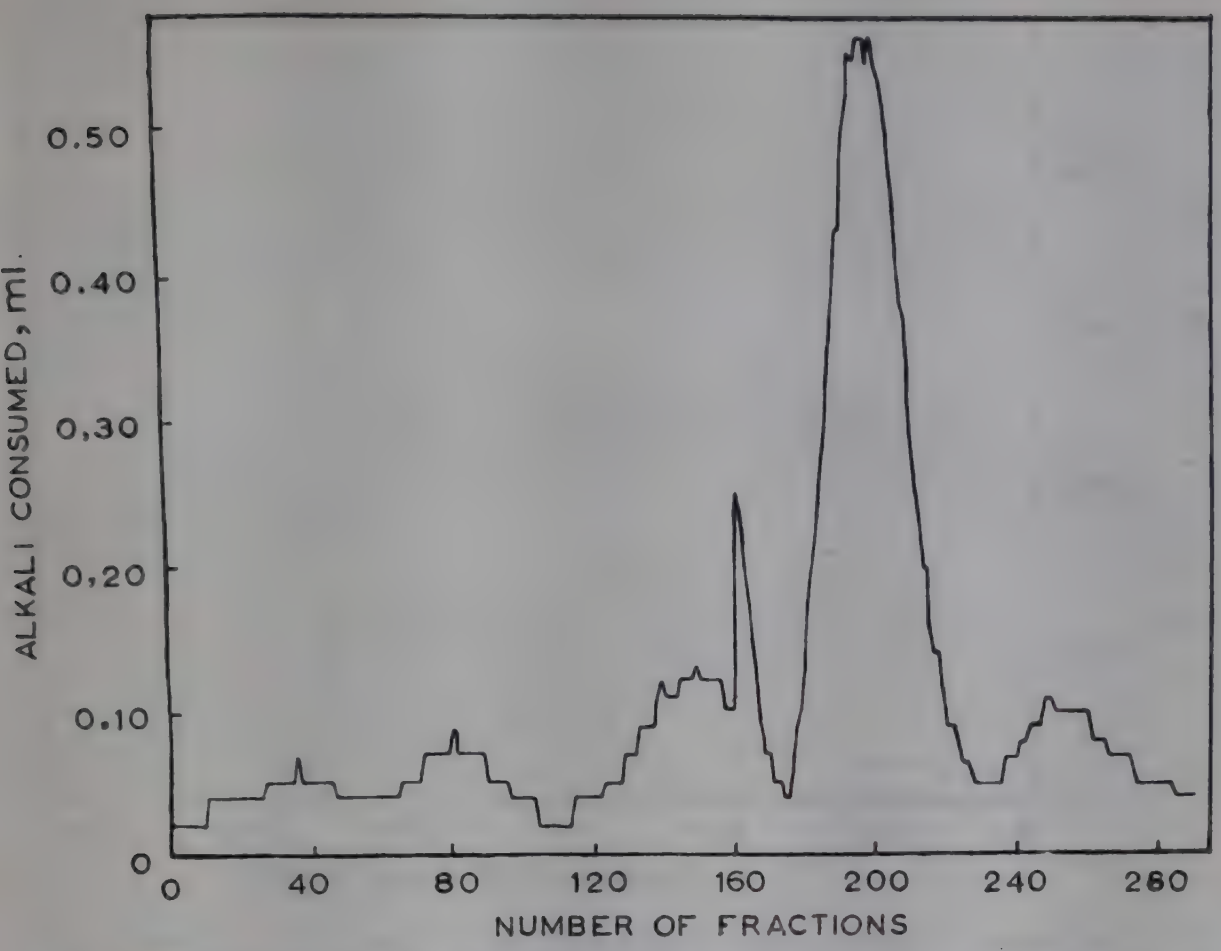


FIG. 6—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF VANASPATI

Azelaic, sebacic and suberic acids were identified after recovery and crystallisation from the respective fractions, as described earlier. Azelaic and sebacic acids were further characterised by the preparation of their *p*-bromophenacyl esters. From the fractions pooled together from a number of runs, a sample of the C_{11} acid with a satisfactory melting point was obtained in low yield. It has not so far been possible to isolate the C_{12} acid in a state of sufficient purity.

As a fairly quantitative separation of the mixture of dicarboxylic acids could be achieved by the above method, it should be possible to quantitatively estimate the different iso-oleic acids present in vanaspati. In hydrogenated fats, the Δ^{-8} and Δ^{-10} monoethenoid acids predominate next to the *trans* isomer of oleic acid, viz., elaidic acid. This is in conformity with the findings of Hilditch and Vidyarthi [12]. If it is assumed that the undecanedioic acid present in the mixture of dicarboxylic acids results solely due to the oxidation of vaccenic acid present in the fat, the content of the latter in the sample of vanaspati can be estimated to be of the order of 7 per cent of the total iso-oleic acids content.

Iso-Oleic Acids in Natural Fats

Several instances of the occurrence of isomers of oleic acid in natural fats have been cited earlier. Of these, vaccenic acid has received considerable attention in recent years due to two main reasons. Firstly, vaccenic acid was the first and for a considerable time the only known example

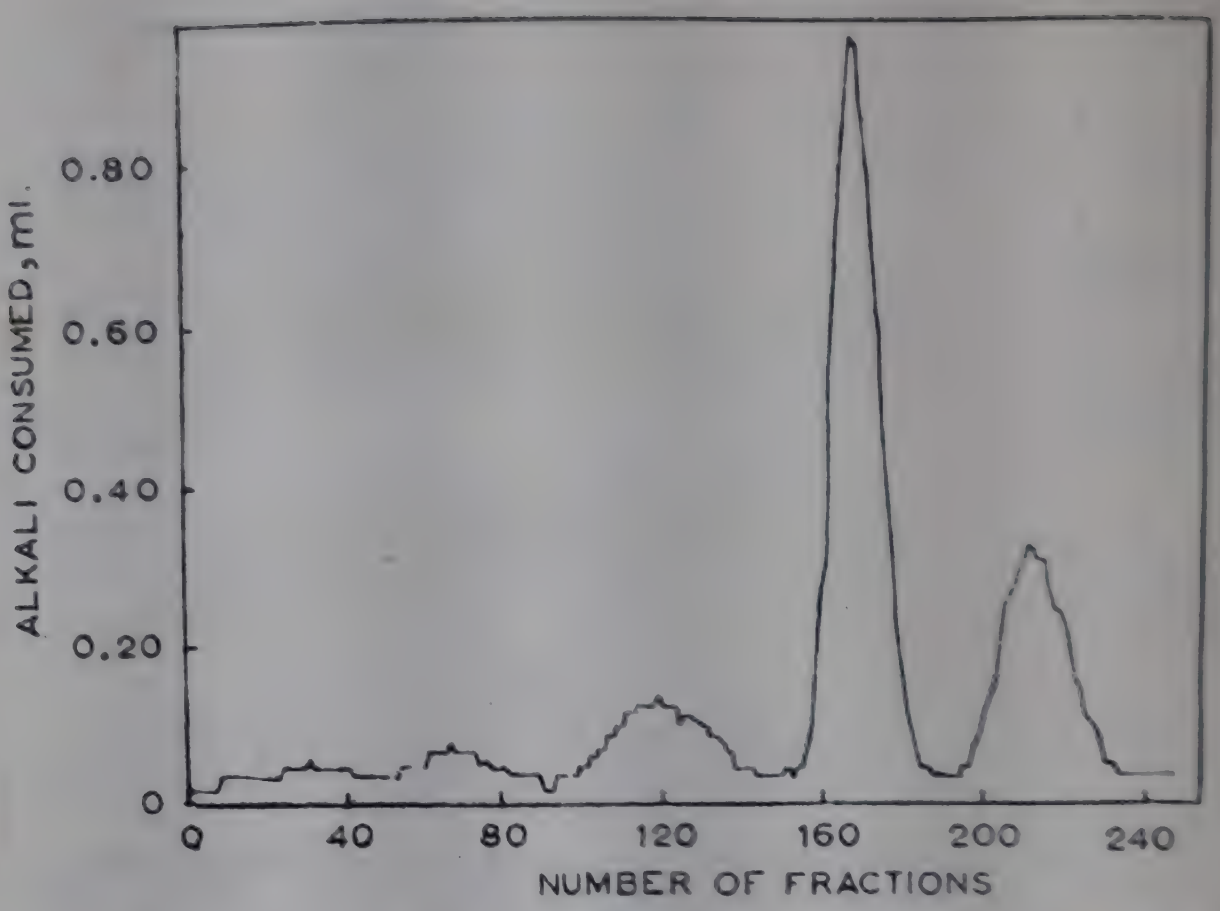


FIG. 7—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF VANASPATI WITH ADDED SUBERIC ACID

of the occurrence of a *trans*-octadecenoic acid. Secondly, one of the earlier reports[19] on the acid had attributed certain unique growth promoting properties to it.

Bertram[3] isolated the acid from the body fats of sheep and oxen, and from butter fat in very small quantities (0.1–1 per cent). The vaccenic acid was assigned the structural formula of *trans*-octadec-11-enoic acid for the following reasons. Ozonolysis of vaccenic acid and oxidation of the aldehyde and aldehydo acid thus obtained led to the formation of a monocarboxylic acid and a dicarboxylic acid, m.p. 109°. The dicarboxylic acid did not depress the melting point of an authentic specimen of nonanedicarboxylic acid. This acid established the presence of the double bond at the 11 position in the original acid. The evidence in favour of a *trans* configuration was the melting point of the acid and its inability to be transformed to the higher melting acid or elaidic form by isomerising agents. Begemann *et al.*[14] who examined chromatographically the dicarboxylic acid fraction produced by the oxidation of a sample of vaccenic acid from butter fat, were also of the opinion that nonanedicarboxylic acid was obtained exclusively as required by Bertram's structure[3].

The purity of vaccenic acid obtained from natural sources has been questioned by several workers. Thus, Gupta, Hilditch, Paul and Shrivastava[20] employed potassium permanganate oxidation of vaccenic acid in acetone solution and found that the dicarboxylic acid fraction resulting from oxidation was a difficultly separable mixture with an indefinite

melting point. They isolated a small quantity of nonanedicarboxylic acid only in one instance. Again, by distilling the monocarboxylic acid produced during oxidation through a fractionating column, they observed consistent evidence for the presence of both *n*-heptanoic and *n*-octanoic acids. Taking all these experimental data into consideration, they suggested that vaccenic acid was a mixture of more than one of the higher *trans* ethenoic fatty acids. Bumpus, Taylor and Strong [21] confirmed the structure of synthetic *trans*-octadec-11-enoic acid by degradation and suggested that the differences in the X-ray diffraction properties of the synthetic *trans*-octadec-11-enoic acid and natural vaccenic acid were due to the presence of isomeric acids in the natural material.

Although the non-homogeneity of vaccenic acid isolated from natural sources has been established by several workers, no quantitative data regarding the composition of the solid iso-oleic acid fraction of butter and beef body fats—two of the reputedly rich sources of vaccenic acid—are as

TABLE 6—DICARBOXYLIC ACIDS IN OXIDATION PRODUCTS OF BEEF BODY FAT ISO-OLEIC ACIDS

(Wt. of silica gel, 35 g. ; wt. of acid mixture, 86.8 g. ; vol. of neutral eluate, 55 ml.)

Acid	Fraction	Alkali (0.031N)R consumed *ml.*	Composition *mg.*
Tridecanedioic	18–56	4.21	19.00
Dodecanedioic	57–70	0.95	4.86
Undecanedioic	71–110	5.33	21.33
Sebacic	112–131	1.47	5.51
Azelaic	132–156	6.78	23.66
Suberic	157–199	2.06	6.65

TABLE 7—DICARBOXYLIC ACID IN OXIDATION PRODUCTS OF BEEF BODY FAT ISO-OLEIC ACIDS AFTER PETROLEUM ETHER TREATMENT

(Length of column, 18 cm. ; wt. of silica gel, 35 g. ; wt. of acid mixture, 81.2 mg. vol. of neutral eluate, 80 ml.)

Acid	Fraction	Alkali (0.031N) consumed *ml.*	Composition *mg.*
Tridecanedioic	1–36	3.87	14.43
Dodecanedioic	38–50	1.07	4.67
Undecanedioic	51–84	6.84	22.45
Sebacic	85–115	1.96	6.04
Azelaic	166–157	7.96	22.83
Suberic	158–180	2.04	5.42

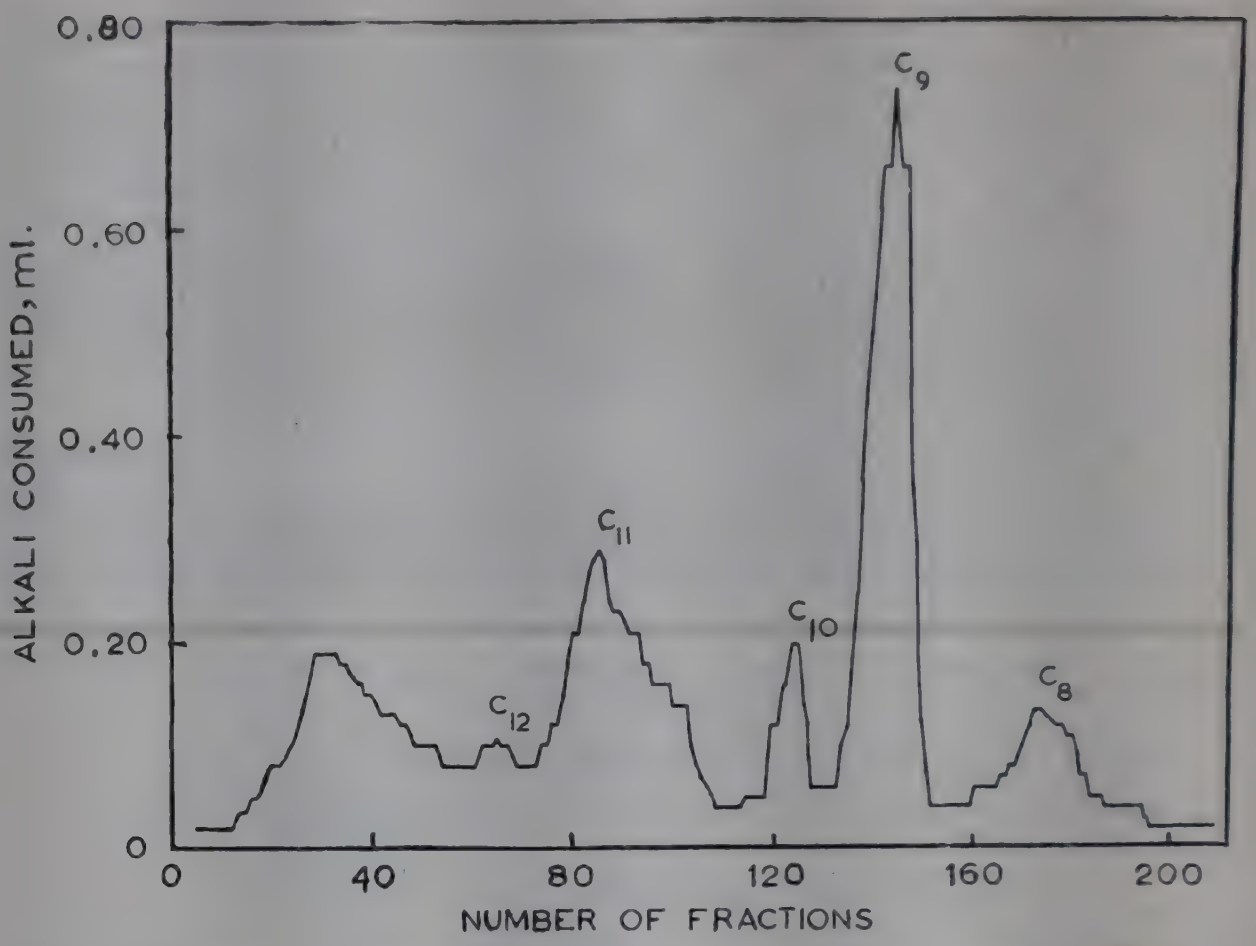


FIG. 8—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF BEEF BODY FAT

yet available. The quantitative examination of the *trans* unsaturated acids of these natural fats, employing the methods described earlier, was, therefore, undertaken.

Beef Body Fat

Solid iso-oleic acids from beef body fat were prepared according to Bertram's procedure[3]. Thus, from 400 g. of mixed fatty acids of beef body fat (iod. val., 29.37), 3.4 g. of solid unsaturated acids (iod. val., 77.0) were obtained. From 1.5 g. of acids, 1.33 g. of methyl esters were obtained. This on oxidation gave 0.75 g. of dicarboxylic acids.

Fig. 8 (Table 6) depicts the results of the chromatographic run of the mixture of dicarboxylic acids obtained by the oxidation of the iso-oleic acid fraction (iod. val., 77.0). The possibilities of the acid represented by the first peak being an impurity of monocarboxylic acid was ruled out by the fact that the peak was also obtained after the mixture had been extracted twice with petroleum ether (Fig. 9, Table 7). The volume of neutral eluate collected prior to the appearance of the peak was also more than that in the case of monocarboxylic acids. As a confirmatory measure, known amounts of palmitic and suberic acids were added to the mixture of dicarboxylic acids which had been treated with petroleum ether and the resultant mixture was separated in the column employing the usual conditions. The addition of suberic acid incidentally confirmed the identities of the dicarboxylic acids. The results obtained

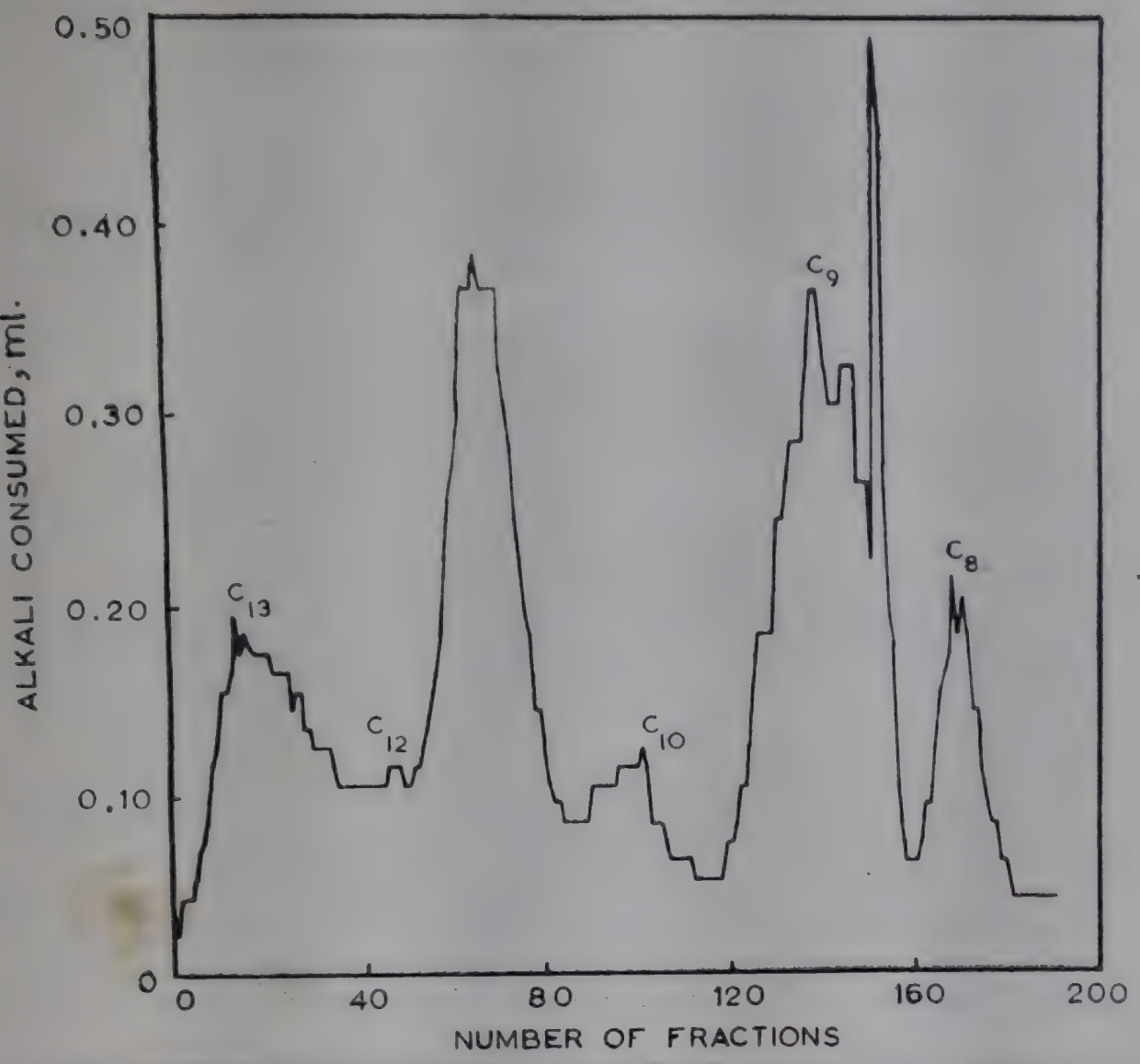


FIG. 9—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF BEEF BODY FAT, AFTER PETROLEUM ETHER TREATMENT

are shown in Fig. 10 (Table 8). It will be seen from the figure that palmitic acid gets eluted as a distinct peak which is absent in Fig. 9. Based on the composition of the dicarboxylic acid mixture as computed from Fig. 9, the recovery of suberic acid (Fig. 10) amounted to 101.6 per cent. The initial peaks in Figs. 9 and 10 are, therefore, to be ascribed to tridecanedioic acid. The composition of the iso-oleic acid fraction of beef body fat as computed from Fig. 8 is as follows: $\triangle^{-13}$, 22.7 per cent; $\triangle^{-12}$, 5.1 per cent; $\triangle^{-11}$, 26.6 per cent; $\triangle^{-10}$, 6.9 per cent; $\triangle^{-9}$, 29.5 ; $\triangle^{-8}$, 8.3 per cent.

Cow Butter Fat

The solid unsaturated acids of cow butter fat were isolated in the same manner as those of beef body fat. Thus, from 618 g. of mixed fatty acids (iod. val., 34.41) was isolated 2.72 g. of solid unsaturated acids (iod. val., 76.8).

The dicarboxylic acid mixture obtained from the iso-oleic acid fraction of cow butter fat gave the chromatographic pattern depicted in

Fig. 11 (Table 9). The first peak in Fig. 11 is again to be ascribed to tridecanedioic acid and not to an impurity of monocarboxylic acid, since it persisted even after the mixture had been extracted twice with hot petroleum ether (Fig. 12, Table 10). The total recovery of acids (Fig. 11) is somewhat lower than usual, because of the fact that the small amounts of dodecanedioic and sebacic acids which have not been eluted in well defined peaks have not been taken into consideration. Excluding $\triangle^{-12}$ and $\triangle^{-10}$ octadecenoic acids, which it has not been possible to estimate with any reasonable accuracy, the composition of the solid iso-oleic acid fraction of cow butter fat as computed from Fig. 11 is as follows: octadecenoic acids: $\triangle^{-13}$, 20.5 per cent; $\triangle^{-11}$, 49.3 per cent; and $\triangle^{-9}$, 17.4 per cent.

The results of studies on iso-oleic acids of natural fats point to the conclusion that in addition to *trans*-octadec-11-enoic acid, other *trans* isomers of oleic acid exist in cow butter and beef body fats. This conclusion conflicts with the views of Bertram and Begemann *et al.* In the present investigation, the unsaturated solid fatty acids were prepared under conditions almost identical to those of Bertram[3] and yet, the dicarboxylic acid fraction resulting from their oxidation was not restricted to the presence of undecanedioic acid alone. It is likely that as Begemann *et al.*[4] worked with small quantities, the minor constituents would have missed their observation. This indirectly brings out the advantages of working with larger amounts (100 mg.), which is possible under the experimental conditions followed in the present investigation. The results of the present investigation are in conformity with the findings of Gupta *et al.*[20] and Bumpus *et al.*[21] and definitely establish the presence of isomeric *trans* octadecenoic acids in beef body and cow butter fats and the fact that the vaccenic acid obtained from natural sources is not a homogeneous substance. Of the octadecenoic acids identified in natural fats,

TABLE 8—DICARBOXYLIC ACIDS IN OXIDATION PRODUCTS OF BEEF BODY FAT ISO-OLEIC ACIDS AFTER PETROLEUM ETHER TREATMENT WITH ADDED PALMITIC AND SUBERIC ACIDS

(*Length of column, 48 cm.; wt. of silica gel, 35 g.; wt. of acid mixture, 82.4 mg.; wt. of added palmitic acid, 2 mg.; wt. of added suberic acid, 11.6 mg.; vol. of neutral eluate, 64 ml.*)

Acid	Fraction	Alkali (0.031N) consumed *ml.*	Composition *mg.*
Palmitic	1–15	1.59	12.31
Tridecanedioic	16–49	4.13	15.24
Dodecanedioic	50–61	1.01	3.51
Undecanedioic	62–94	6.85	22.38
Sebacic	95–124	1.84	5.62
Azelaic	128–163	9.05	25.72
Suberic	164–188	6.73	17.71

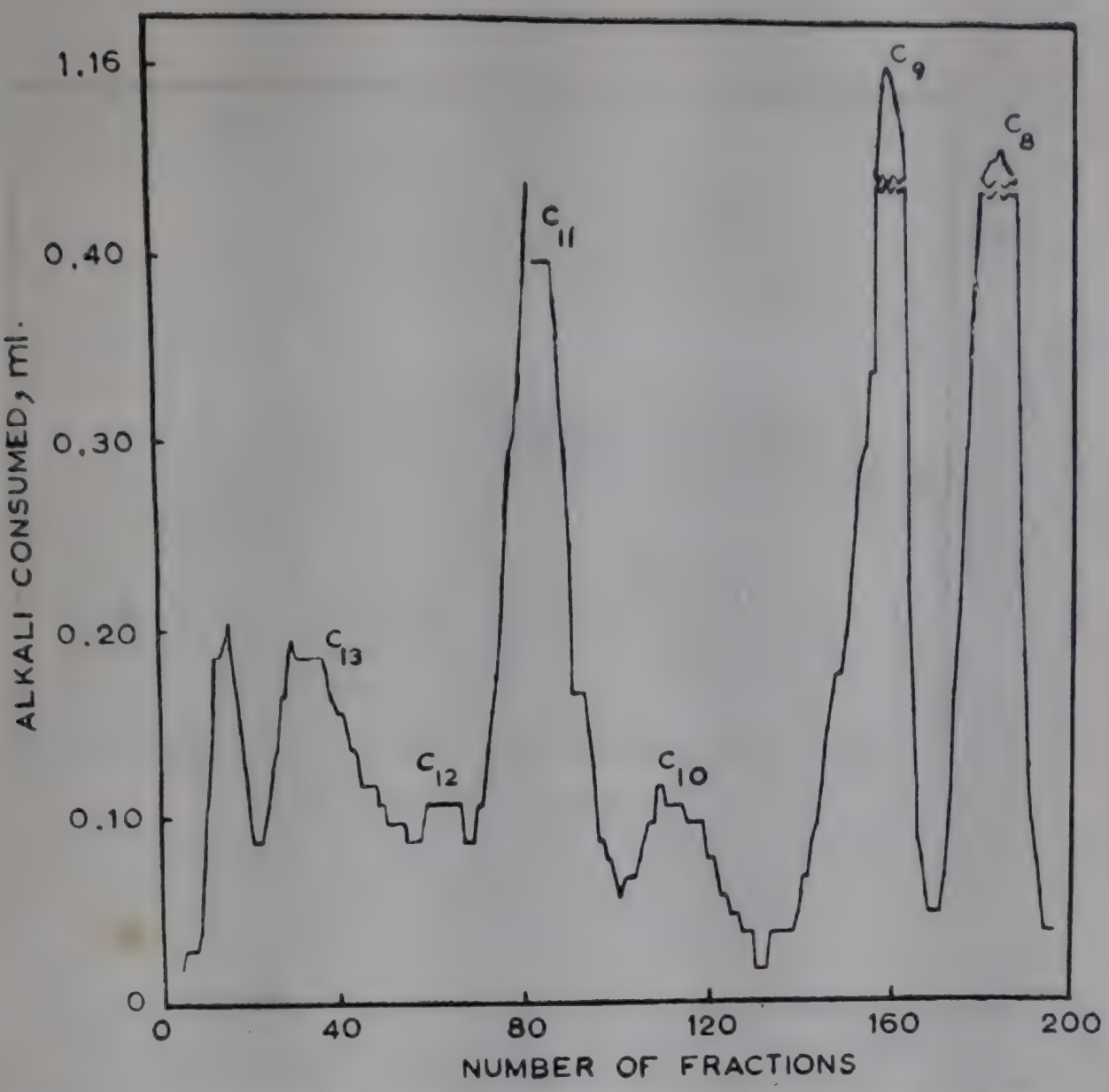


FIG. 10—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF BEEF BODY FAT AFTER PETROLEUM ETHER TREATMENT WITH ADDED PALMITIC AND SUBERIC ACIDS

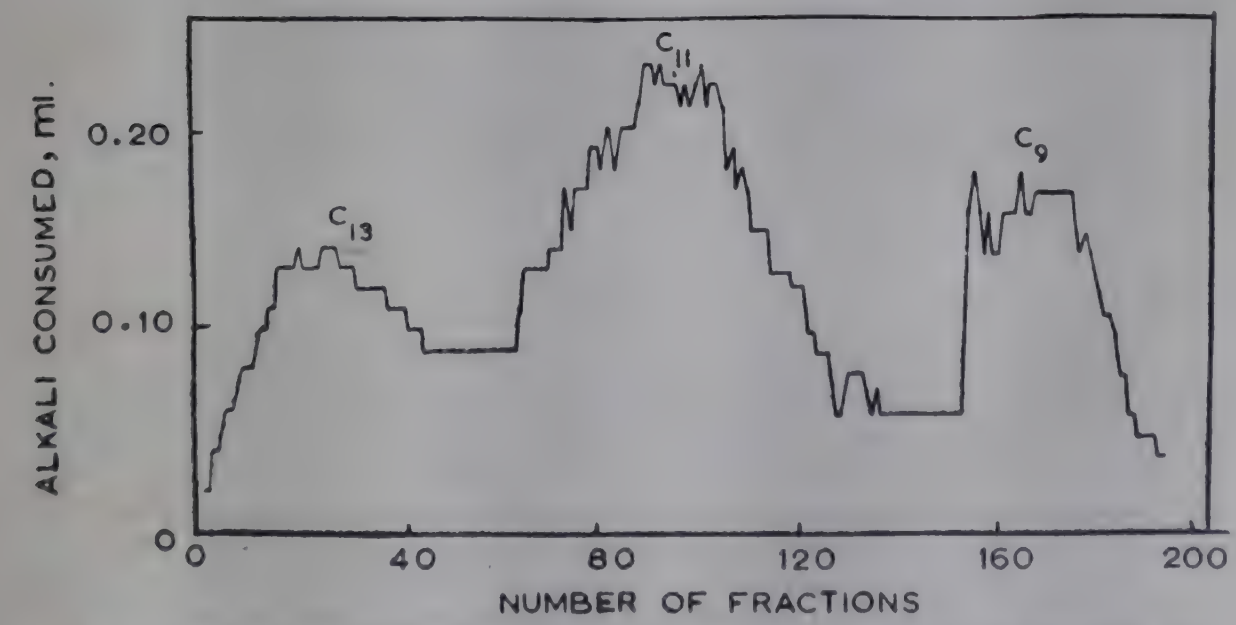


FIG. 11—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF COW BUTTER FAT

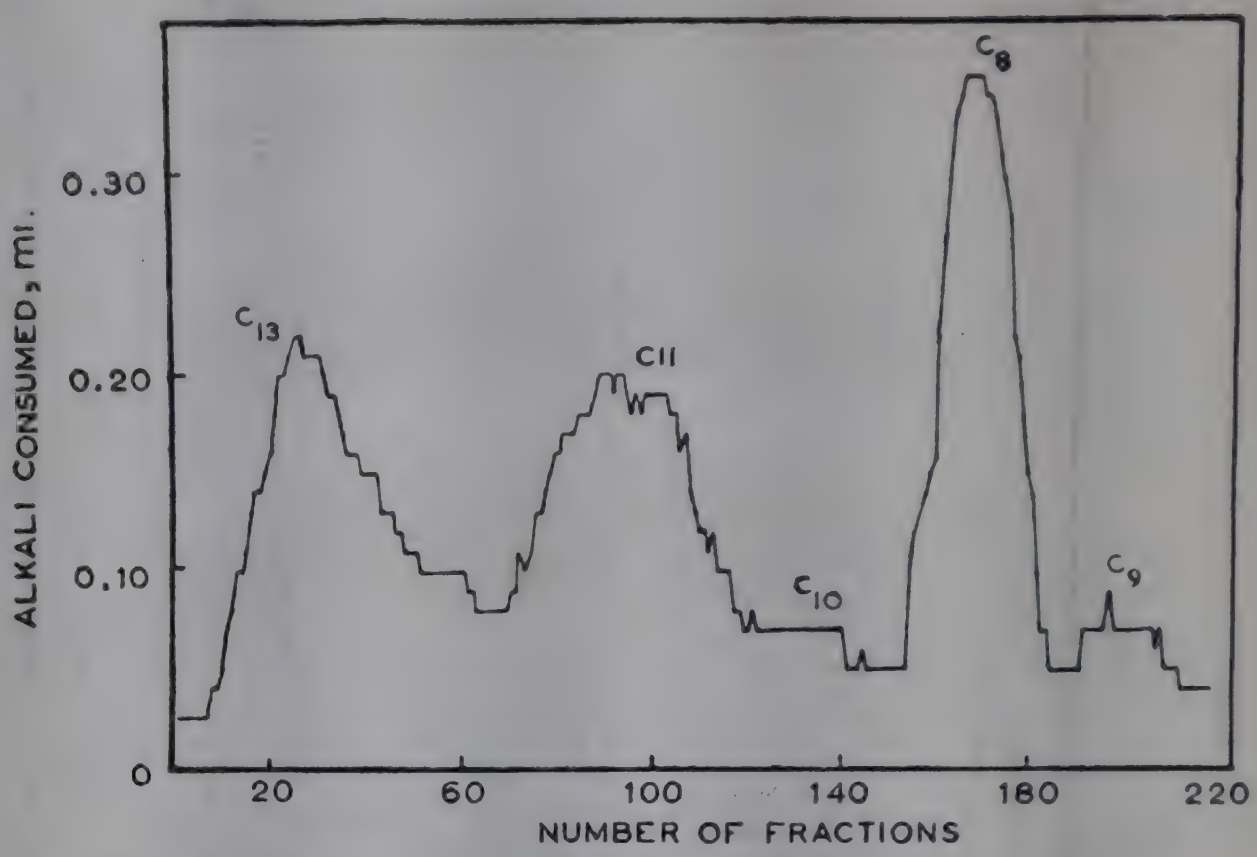


FIG. 12—CHROMATOGRAPHIC RUN OF A MIXTURE OF DICARBOXYLIC ACIDS OBTAINED ON OXIDATION OF ISO-OLEIC ACIDS OF COW BUTTER FAT, AFTER PETROLEUM ETHER TREATMENT

trans-octadec-11-enoic and *trans*-octadec-10-enoic acids have already been identified[20]. The results of Swern, Knight and Eddy[22] would indicate the presence of *trans*-octadec-9-enoic acid in some natural fats. These acids would give rise to undecanedioic, sebacic and azelaic acids on oxidation with potassium permanganate. The C_{13} and C_{12} dicarboxylic acids are not likely to be derived from the naturally occurring diethenoid acids, for it has been shown that the diethenoid acids on oxidation yield only azelaic acid[23]. Whether they can be so derived from C_{20}–C_{22} unsaturated acids, the presence of which in butter fat has been demonstrated by Shorland and Johanneson[24], is not quite certain. The present evidence would, however, point to the positional isomers of octadecenoic acids being the possible source of these dicarboxylic acids.

SUMMARY

Fractions rich in iso-oleic acids have been obtained from the mixed fatty acids of vanaspati and beef body fat by fractional crystallisation of the urea adducts from methanol. The enrichment effected compares favourably with that resulting from lead salt separation. In order to keep the crystallisation steps to the minimum, the mercuric salt fractionation was used for the separation of saturated and *trans* unsaturated acids.

Paper chromatography of a mixture of dicarboxylic acids leads to their facile separation. The method has been employed for the study of the iso-oleic acid fraction in hydrogenated fat and cow butter fat.

Quantitative separation and estimation of the iso-oleic acid fractions of vanaspati, beef body and cow butter fats have been carried out employing partition chromatography.

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III

Studies in the Processing and Utilization of Cottonseed Oil for Edible Purposes with a Note on the Composition, Properties and Nutritive Value of Palm Oil

M. Narayana Rao, S. Kuppuswamy, K. Krishnamurthy, D.S. Bhatia, M. Swaminathan & V. Subrahmanyan

Realising the need for exploring the possibility of extending the use of cottonseed oil for edible purposes in India, the *Vanaspati Research Advisory Committee* of the Council of Scientific & Industrial Research sanctioned funds for a scheme of research on the processing and utilization of cottonseed oil for edible purposes, to be carried out at the Central Food Technological Research Institute, Mysore. The scheme started functioning in May 1952 and continued for a period of about 5 years. There were occasional breaks due to change of personnel, but the main programme was carried out. The papers which follow relate to a substantial part of the work carried out at Mysore from May 1952 to Feb. 1957.

An extensive programme of work on the production and processing of rice oil (also known as rice bran oil) was also carried out at Mysore. Rice oil would be a useful raw material for vanaspati manufacture if oil of good quality can be produced in sufficient quantities. As this work was not done exclusively under the auspices of the *Vanaspati Research Advisory Committee*, it has not been included in the present series. Some investigations were also carried out on the utilization of low-fat cottonseed flour, but as they are not of direct interest to vanaspati industry, the related papers have not been included.

1. COMPOSITION OF CERTAIN VARIETIES OF COTTONSEED AND REFINING AND BLEACHING OF CRUDE COTTONSEED OIL

Crude cottonseed oil, obtained by expressing steamed cottonseed kernels, has a dark brown colour and contains gossypol which is toxic. Consequently, unlike groundnut, coconut and gingelly oils, cottonseed oil cannot be used for edible purposes without preliminary refining. In any programme for the utilization of cottonseed oil, data on the physical and chemical composition of cottonseeds grown in the country should be a primary requisite. Hence, a study of the chemical composition of nine varieties of cottonseed and refining and bleaching of crude cotton-

TABLE 1—PROPORTION OF LINTER, HULL AND KERNEL IN DIFFERENT COTTONSEEDS

Variety	Size	Seed index *g./100/g.*	Linter %	Hull %	Kernel %
Lakshmi (Gadag)	Big	8.47	12.8	38.2	49.0
Indore No. 2 (Indore)	Big	8.63	13.8	39.5	46.7
C 520 (Shahar)	Big	8.03	13.0	36.2	50.8
Kalyan (Vighaon)	Medium	7.50	12.0	41.6	46.4
Vijaya (Broach)	Medium	6.29	11.5	44.1	44.4
Navapur (W. Khandesh)	Small	5.85	10.1	43.2	46.7
Nander (C. Rly.)	Small	5.85	10.6	43.2	46.2
Malvi No. 7 (Indore)	Small	4.96	9.8	43.2	47.0
Jalgaon (E. Khandesh)	Small	4.83	9.2	44.4	46.4
American varieties in USA[5]		10–11	11–17	36–43	44–52

TABLE 2—MOISTURE, PROTEIN AND FAT CONTENT OF COTTONSEED AND SEED-KERNEL

Variety	Moisture, %		Protein, % (N×6.25)		Fat (ether extractives), %	
	Whole seed	Kernel	Whole seed	Kernel	Whole seed	Kernel
Lakshmi (Gadag)	8.90	6.7	20.90	33.1	19.40	35.2
Indore No. 2 (Indore)	10.04	7.8	17.92	29.2	18.95	33.2
Kalyan (Virghaon)	9.96	6.9	16.87	27.4	16.50	30.5
C 520 (Shahar)	6.70	6.0	17.65	29.4	18.30	34.1
Vijaya (Broach)	10.70	7.9	18.84	30.2	15.15	33.1
Navapur (W. Khandesh)	8.10	6.7	17.52	29.4	13.95	28.9
Nander (C. Rly.)	7.90	6.2	19.90	32.9	17.40	32.1
Malvi No. 7 (Indore)	10.60	7.8	18.10	32.5	15.50	30.6
Jalgaon (E. Khandesh)	7.70	6.1	19.10	32.1	17.20	32.6
American varieties in U.S.A.[5]	6.7–7.9	5.6–7.1	20.0–23.0	31.1–37.1	18.0–29.0	31.9–36.6

seed oil obtained from them was undertaken. Since the completion of the work reported here, results of two studies on Indian cottonseed were published[1,2].

Materials and Methods

Representative samples of nine varieties of cottonseed grown in the major cotton-growing states of India were obtained through the Agricultural Departments of the respective states.

In the absence of a delinting machine, the seeds were delinted by means of a rice huller (*Batliboi Ltd.*, Sun Brand) which gave satisfactory results. For dehulling, the seeds were broken in a disintegrator (*Christy & Norris* Laboratory Mill, size 8 in.) using 12 mesh sieve and the broken mass was sifted through a 20 mesh sieve. Data regarding the proportion of linters, hulls and kernels in different varieties are given in Table 1.

Whole cottonseed and kernels were analysed for moisture, nitrogen and fat, according to the methods of the Association of Official Agricultural Chemists[3]. The results are summarized in Table 2.

Refining and bleaching of cottonseed oil: Crude cottonseed oil obtained by expressing steamed cottonseed kernels was dark in colour and had an acid value of 2.5. The oil was degummed by washing with water to remove the impurities, such as phosphatides and gummy or mucilaginous substances. This step was necessary to reduce the refining loss. The degummed oil was refined according to the official methods of the American Oil Chemists' Society[4]. For re-refining the oil, 750 g. of the refined oil was treated with 6 ml. of 25 per cent sodium hydroxide and was agitated at 1500 r.p.m. at 50°C. for 15 min. After standing overnight, the oil was decanted and washed with hot water until free from soap. The acid values of refined and re-refined cottonseed oil were 0.15 and 0.05 respectively. For bleaching the refined and re-refined cottonseed oil, fuller's earth, animal charcoal and saw dust charcoal (prepared in the laboratory from saw dust obtained from rosewood after activation with zinc chloride), were tried at various levels. The procedure adopted was the same as that recommmended by A.O.C.S.[4] After bleaching, the oil was filtered and the colour of the oil determined by a Lovibond tintometer. The results are given in Table 3.

Gossypol content of cottonseed and cottonseed oil: The effect of genetic and environmental factors on the composition of cottonseed and the properties of lint have been the subject of numerous investigations[5 6]. Comparatively less attention has so far been paid to the effect of these factors on the gossypol content of cottonseed and cottonseed oil[7]. A major proportion of the yellow pigments present in the seed and the crude oil is contributed by gossypol which is a toxic principle[8]. In the present investigation, the gossypol content of nine varieties of Indian cottonseed and the crude oil obtained from them has been determined. The effect of refining on the gossypol content of crude cottonseed oil has also been studied.

The kernel from whole cottonseed was prepared according to the procedure already described. Crude cottonseed oil was obtained by steaming the kernels for half an hour and then expressing in a hydraulic press under a pressure of 1.5 tons per sq. in. The crude oil was refined and re-refined as described earlier. The free gossypol present in the whole seed, kernel and crude and refined oils was estimated according to the procedure of Pons and Guthrie[9 10], using pure gossypol (m.p. 184°C.) prepared from raw cottonseed kernels as standard by the method of Murthy *et al.*[11] The results are given in Table 4.

TABLE 3—COLOUR OF REFINED AND RE-REFINED COTTONSEED OIL

(Colour in Lovibond Scale, 1 cm. cell ; colour of untreated oil : refined, 10.0 R & 19.9 Y; re-refined, 2. 2R & 8.0Y)

Decolorizing agent	Refined		Re-refined	
	Red	Yellow	Red	Yellow
Fuller's earth, 1%	9.9	15.5	2.1	7.0
do 2%	9.9	15.5	2.1	6.0
do 3%	8.1	14.4	1.7	5.0
do 4%	8.4	14.4	1.9	6.0
do 5%	7.2	12.2	1.6	5.0
Animal charcoal, 1%	6.0	13.0	1.0	6.0
do 2%	5.8	10.0	1.0	5.0
Fuller's earth, 1% + animal charcoal, 1%	6.0	10.0	1.2	6.0
Fuller's earth, 2% + animal charcoal, 2%	5.8	9.0	1.1	5.0
Sawdust charcoal, 1%	7.0	14.4	1.4	5.0
do 2%	6.0	13.3	1.3	5.0
Fuller's earth, 1% + sawdust charcoal, 1%	6.6	15.2	1.0	5.1
Fuller's earth, 2% + sawdust charcoal, 2%	5.5	9.0	1.0	5.0
Fuller's earth, 3% + sawdust charcoal, 3%	3.8	9.6	0.8	3.8

TABLE 4—GOSSYPOL CONTENT OF COTTONSEED AND CRUDE COTTONSEED OIL

Variety	Whole seed %	Kernel %	Crude oil %
Lakshmi (Gadag)	0.83	1.82	0.41
Indore No. 2 (Indore)	0.80	1.72	0.42
C *250* (Shahar)	0.81	1.64	0.45
Kalyan (Virghaon)	0.91	2.32	0.47
Vijaya (Broach)	0.72	1.75	0.39
Navapur (W. Khandesh)	0.92	2.35	0.45
Nander (C. Rly)	0.73	1.56	0.38
Malsi No. 7 (Indore)	0.72	1.66	0.42
Jalgaon (E. Khandesh)	0.81	2.22	0.40
American varieties in U.S.A.	0.8-1.5	1.8-2.6	0.4-0.48

Discussion

The differences in the composition of the nine varieties of Indian cottonseed grown in different parts of India may be due to varietal and/or environmental factors. From the results given in Table 2 it will be clear that the fuzzy varieties, *Lakshmi* (Gadag), *Indore* No. 2 (Indore), C 520 (Shahar), and *Kalyan* (Virghaon), contain higher percentages of both oil and lint than the *desi* ones. The fuzzy varieties can therefore be used economically in the cottonseed oil industry. It is found that the bleaching action of fuller's earth is effectively improved by using it in combination with 2 per cent animal or saw dust charcoal (Table 3).

The gossypol content of Indian cottonseed is low (0·7–0·92 per cent) when compared to Egyptian and Sea Island varieties (1·9–3·0 per cent)[7]. Refining of cottonseed oil completely removes the gossypol.

2. DIGESTIBILITY OF CRUDE, REFINED AND HYDROGENATED COTTONSEED OIL

In addition to its use as a salad oil, cottonseed oil is also hydrogenated and used in the preparation of shortening and margarine[5]. Although a large amount of work has been done on the digestibility and nutritive value of a wide variety of edible oils[12–15] little information is available concerning crude, refined and hydrogenated cottonseed oil. The present paper deals with studies on the digestibility of these fats as compared with refined groundnut oil and cow's ghee.

Experimental Procedure and Results

Materials: Crude cottonseed oil used in this study was prepared as follows: Healthy cottonseeds were first delinted in a rice huller (*Batliboi Ltd.*, Sun Brand) and then broken up in a disintegrator (*Christy & Norris* Laboratory mill, size 8 in.) using a 12 mesh sieve and the broken mass was sifted through a 20 mesh sieve. The kernels thus obtained were steamed for an hour and expressed in a hydraulic press under a pressure of 1.25 tons per sq. in. The crude cottonseed oil was dark brownish in colour and had an acid value of 2.5.

Refined cottonseed oil was prepared by refining and re-refining the crude oil according to the procedure of Narayana Rao and Kuppuswamy[16]. Hydrogenated cottonseed oil (m.p. 37°C.) was supplied by *Hindustan Vanaspati Manufacturing Company*. A sample of refined groundnut oil was purchased from a local oil mill. A genuine sample of cow's ghee (clarified butter fat) was obtained through the courtesy of the Indian Dairy Research Institute, Bangalore. All the fats and oils were fresh and pure. The physical and chemical constants of the fats and oils were determined according to A.O.A.C. methods[7]. The results are given in Table 5.

In vitro **digestibility:** The rates of digestion of the different fats were studied using both castorseed and pancreatic lipases.

The castorseed lipase was prepared from the seeds of (*Ricinus communis* Linn.) by the method of Longnecker and Haley[17]. The procedure adopted for studying the *in vitro* digestibility was the same as that describ-

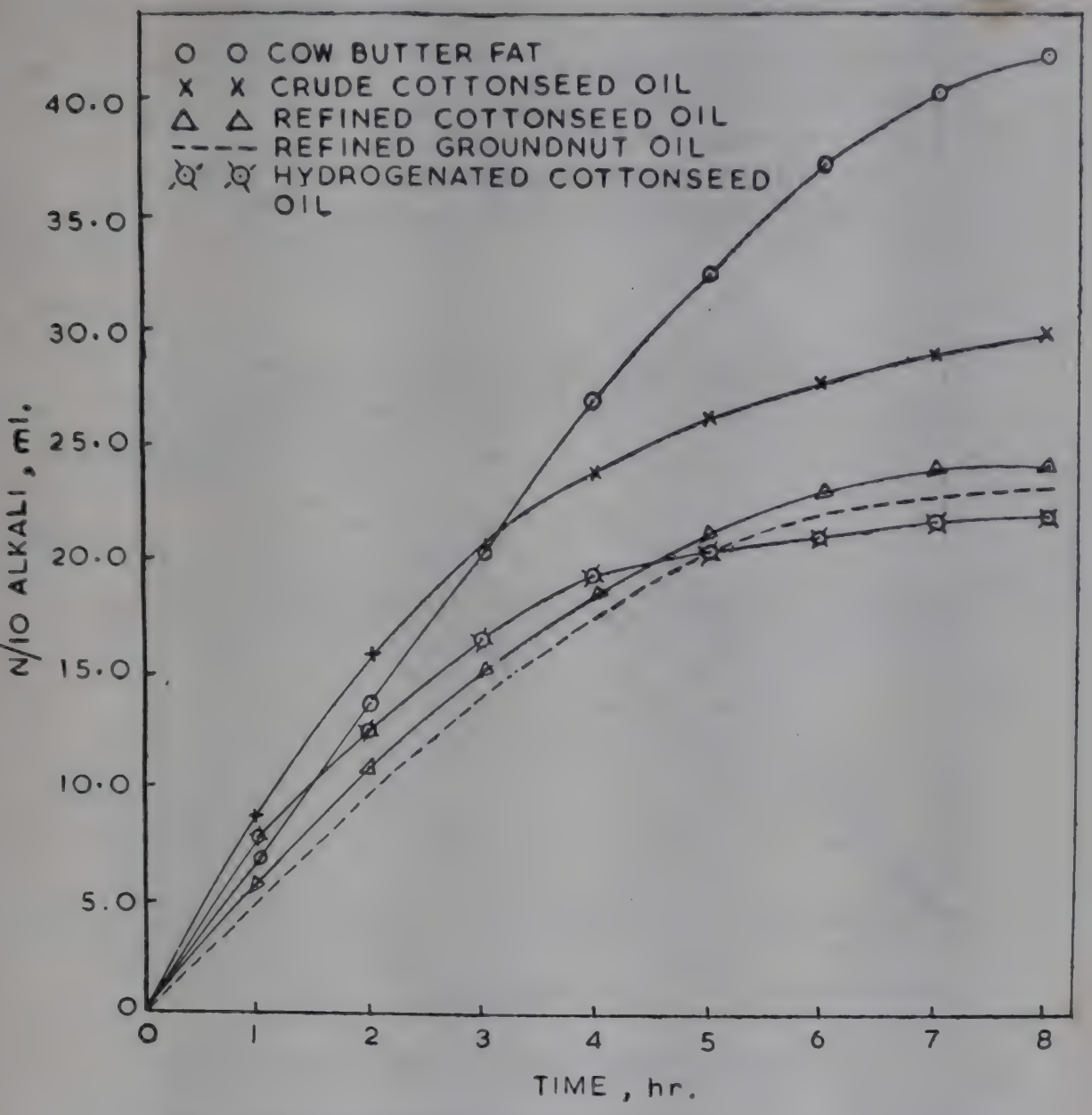

FIG. 1—RATE OF HYDROLYSIS OF FATS USING CASTOR SEED LIPASE

ed by Dastur and Giri[18]. The relative rates of hydrolysis of different oils and fats are represented graphically in Fig. 1.

Pancreatic lipase was prepared from fresh pigs' pancreas according to the procedure of Willstatter and Waldschmidt Lietz [19]. The digestion of fats was followed up by the method outlined by Weinstein and Wynne[10]. The fats were emulsified with gum acacia and egg albumin in the presence of ammonia-ammonium chloride buffer (*p*H 8.9) and used as substrates. The quantity of fatty acid released at various intervals of time on incubation at 37°C. with an extract of the enzyme in 50 per cent glycerol was determined by titration against N/10 alkali using phenolphthalein as indicator after the addition of neutralized alcohol-ether mixture (5:1). The relative rates of hydrolysis of the five fats studied are represented graphically in Fig. 2.

In vivo **digestibility:** The digestibility coefficients of the fats were determined using adult rats fed diets containing different fats at 10 per cent level adopting a method similar to that of Misra and Patwardhan. Table 6 gives the composition of diets used in this study. Two drops

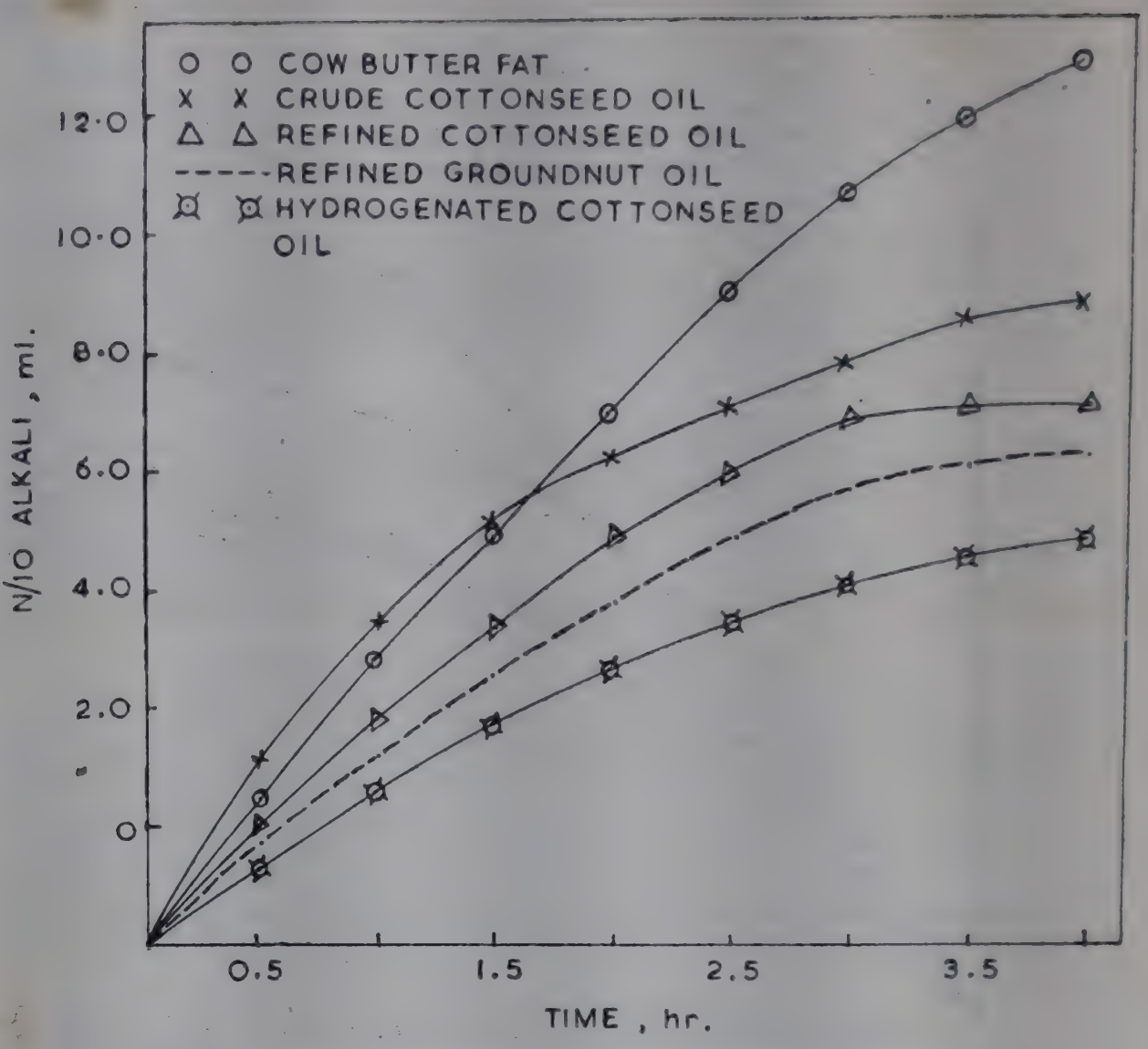

FIG. 2—RATE OF HYDROLYSIS OF FATS USING PANCREATIC LIPASE

of Adexoline and 2 mg. of alpha-tocopherol were also given orally to each rat twice a week to meet the requirements of vitamins A, D and E.

Five adult male albino rats from the stock colony, weighing between 130-140 g., were selected for the experiment. They were housed in individual metabolism cages provided with raised screen bottoms and a special feeding device whereby scattering of the diet and contamination of the diet with excreta were minimised. To begin with, they were fed a fat-free diet having a composition similar to that of experimental diets (Table 6) with the difference that the fat was completely replaced by corn starch. Following a preliminary feeding period of 5 days, the feces were collected for 10 days. The 'metabolic fat' excreted by each animal was determined in the usual manner by drying the feces and estimating the total fat according to the procedure of Narayana Rao and De[22]. The results are given in Table 7.

The same rats were then fed the experimental diets containing fats at 10 per cent level (Table 6). The experiment was so designed that each of the five rats received a different fat. When the first period was over, the fat in the diet of each rat was changed. This arrangement was followed till all the five rats had received each one of the fats in turn.

TABLE 5—PHYSICAL AND CHEMICAL CONSTANTS OF FATS EXAMINED

Fat	m.p. °C.	Sap. val.	Iod. val.	Thiocyanogen val.	Acid val.
Crude cottonseed oil	Liquid at room temp.	188.0	100.5	59.0	2.05
Refined cottonseed oil	do	194.5	102.6	60.3	0.58
Hydrogenated cottonseed oil	37	189.0	69.0	..	0.58
Refined groundnut oil	Liquid at room temp.	188.0	90.6	62.0	0.05
Cow's ghee	33	220.0	32.9	26.3	0.21

TABLE 6—COMPOSITION OF EXPERIMENTAL DIETS

	%
Casein (fat-free)	12.0
Corn starch	60.0
Sucrose	10.0
Vitaminised starch*	4.0
Osborne Mendel salt mixture	4.0
Fat†	10.0

*Vitaminised starch was so prepared that at 4 per cent level, it supplied the requirements of all B-group of vitamins †Crude cottonseed oil, hydrogenated cottonseed oil (m.p. 37°C.), refined cottonseed oil, refined groundnut oil and cow's ghee

TABLE 7—METABOLIC FAT OF RATS FED FAT-FREE DIETS

(*Collection period, 10 days*)

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Amount of diet consumed, g.	146.0	134.0	143.0	143.0	137 5
Amount of fat ingested	..	..	..	..	..
Weight of dry feces, g.	7.233	6.413	6.347	7.072	6.90
Total metabolic fat, mg.	390.5	352.8	394.5	367.4	399.8
Metabolic fat*/g., stool, mg.	54.0	55.0	62.1	51.9	58.0

*The metabolic fat is almost the same in all rats. The av. value of 56 mg. agrees with reported values[14 16]

Individual records of diet consumption were maintained. The digestibility coefficients of different fats were calculated in the usual manner after allowing for the metabolic fat. The results are presented in Table 8.

Discussion

The results of *in vitro* studies show that the course of hydrolysis of cow's ghee is significantly different from that of other fats (Figs. 1 and 2). This finding confirms the earlier observations of Narayana Rao and Swaminathan[23] and Dastur and Giri[11]. The hydrolyses of the other fats studied follow nearly the same course. While both crude and refined cottonseed oil are slightly more digestible than refined groundnut oil, hydrogenated cottonseed oil is slightly less digestible. Velluz[24] found that when castor lipase is added to equimolecular mixtures of alcohol and different fatty acids and incubated at 37°C., no esterification occurs with acids

Table 8—Digestibility of Oils and Fats
(*Duration of collection, 10 days*)

	Cow's ghee	Crude cotton-seed oil	Refined cotton-seed oil	Refined ground-nut oil	Hydro-genated cotton-seed oil
Number of rats	5	5	5	5	5
Av. amount of diet consumed, g.	82.60	81.60	83.30	82.00	82.70
Av. amount of fat ingested, g.	8.26	8.16	8.33	8.20	8.27
Av. weight of stools, g.	4.62	4.59	4.50	4.47	4.91
Total amount of fat excreted (corrected), mg.	447.80	245.30	430.60	365.70	540.00
Coefficient of digestibility, %	94.60	97.50	94.80	95.90	93.50

Table 9—Composition of Experimental Diets

Extracted skimmed milk powder*	20
Extracted food yeast*	16
Sugar	10
Vitaminized starch [35]	5
Salt mixture (Ca & P free)[36]	4
Agar agar powder	1
Corn starch	34
Fat	10

*Extracted by petroleum ether in a pilot solvent extraction apparatus

containing less than seven carbon atoms in the molecule. His observation would suggest that the greater digestibility of cow's ghee is probably due to its higher content of lower fatty acids.

The results of *in vivo* studies given in Table 4 indicate that all the fats are digested to the extent of about 95 per cent by adult rats. This is in conformity with the observation of Deuel and Holmes[5] that all fats and oils having a melting point below 50°C. are almost completely digestible. Crude cottonseed oil is slightly more digestible than the refined and hydrogenated oils. This may be due to the presence of lecithin and other phosphatides in crude cottonseed oil, which have a beneficial effect on fat digestibility[25].

3. NUTRITIVE VALUE OF CRUDE, REFINED AND HYDROGENATED COTTONSEED OIL

The nutritive value of fats used for edible purposes has been the subject of study by a large number of workers. Deuel *et al.*[26,27], Parish *et al.*[28] and Harris and Mosher[29] did not find any difference in the growth promoting value of different fats, when fed along with an adequate diet to rats. Schantz *et al.*[30] and Boutwell *et al.*[31] reported butter fat to be superior to other fats in promoting growth in albino rats; the superiority being observed when the carbohydrate present in the diet is mainly lactose[32]. Fats are also found to have a beneficial effect on the utilisation of calcium and phosphorus[33,34]. Although a large amount of work has been done on the nutritive value and effect on mineral metabolism of a wide variety of edible oils, little information is available concerning crude, refined, and hydrogenated cottonseed oil. The present paper deals with studies on the growth promoting value and effect on mineral metabolism of crude, refined and hydrogenated cottonseed oil in growing rats.

Experimental

The different fats used in the present study were the same as those employed in a previous investigation.

Young albino rats, one month old and weighing about 40 g. were used in these investigations. Each group consisted of six rats distributed equally according to sex and litter mates. The different groups were fed diets, the composition of which is shown in Table 9; in addition, two drops of Adexoline (*Glaxo*) were given orally to each rat twice a week to meet their vitamins A and D requirements. The rats were kept in individual cages with raised screen bottoms and fed *ad lib* on the diets. The animals were weighed weekly. The experiment lasted for a period of 6 weeks. Records of daily food intake were maintained. The data are presented in Table 10.

Effect on calcium and phosphorus metabolism (metabolism study): During the third and fourth weeks of feeding all the rats were kept in individual mesh-bottomed metabolism cages, each of which was set on a glass funnel in which was placed a metal screen for the separation of feces and urine. The feces were removed from the screen every day, stored in methanol and kept in a refrigerator. Bottles containing about

TABLE 10—GROWTH PROMOTING VALUE OF DIFFERENT FATS

Fat	Av. initial wt. *g.*	Av. final wt. *g.*	Increase in wt. *g.*	Av. weekly increase in wt. *g.*	Increase in wt./*g.* fat *g.*
Crude cottonseed oil	52.1	144.2	92.1	15.35	2.31
Refined cottonseed oil	52.1	145.0	92.9	15.48	2.31
Cottonseed vanaspati	52.0	138.1	86.1	14.38	2.18
Refined groundnut oil	51.9	138.3	86.4	14.40	2.42
Cow's ghee	52.0	147.9	95.9	15.98	2.41

TABLE 11—EFFECT OF COTTONSEED OILS ON CALCIUM AND PHOSPHORUS METABOLISM

(*Duration of experiment, 7 days*)

	Crude cotton-seed oil	Refined cotton-seed oil	Cotton-seed vanaspati	Refined ground-nut oil	Cow's ghee
No. of rats	6	6	6	6	6
Av. initial wt., g.	92.4	92.9	90.5	88.8	93.4
Av. final wt., g.	120.0	122.0	119.4	118.7	122.8
Av. gain in wt., g.	29.6	29.1	28.9	29.9	29.4
Av. Ca in diet consumed, mg.	368.0	375.8	363.8	348.0	370.8
Av. Ca in urines, mg.	12.18	10.22	11.2	12.04	10.08
Ca in feces, mg.	81.02	66.88	93.9	70.76	52.32
Total Ca excreted, mg.	93.20	77.10	105.1	82.80	62.40
% Ca* utilized	74.7 ± 3.1	79.5 ± 3.4	71.1 ± 2.8	76.2 ± 2.1	83.2 ± 2.9
Av. P in diet consumed, mg.	385.6	393.6	381.2	364.6	388.6
Av. P in urine, mg.	6.44	7.14	8.82	7.42	7.7
Av. P in feces, mg.	121.96	117.66	139.88	141.38	126.7
Total P excreted, mg.	128.4	124.8	148.7	148.8	134.4
% P utilized*	66.7 ± 2.5	68.3 ± 3.4	61.0 ± 3.0	59.2 ± 3.2	65.4 ± 2.6

*Including standard error of mean calculated by the formula $\sqrt{\frac{d^2/n-1}{n}}$ where *d* is the deviation from the mean and *n* is the number

10 ml. of the preservative (equal quantities of toluene and conc. hydrochloric acid) were placed beneath each funnel for collection of urine. After the collection period, the funnels were washed with water, acidified with hydrochloric acid, and the washings were added to the urine in the bottles. The unconsumed diet was collected every day in bottles and weighed after drying in a hot air oven at 100°C. to constant weight. Calcium and phosphorus determinations of the diets and of samples of the feces and urine were made using the methods described by Subrahmanyan *et al.*[35] The results obtained are given in Table 11.

Effect on the retention of calcium and phosphorus (carcass analysis study): At the end of 6 weeks' feeding period, all the rats were sacrificed and their total body calcium and phosphorus was estimated. The gastrointestinal tract was dissected and the contents removed. The whole rat was mashed in a meat mincer and was quantitatively transferred into a porcelain dish. Sufficient alcohol was added and the dish was allowed to dry. It was then ashed first on a low flame and then in the muffle at 500°C. after the addition of sufficient amount of sodium acetate which fixes up the phosphorus and prevents it from volatilization. Calcium and phosphorus were estimated according to the methods of Subrahmanyan *et al.*[36]

For calculating the quantity of calcium retained in the body, it was necessary to make allowance for the initial calcium and phosphorus content of the animals at the beginning of the experiment. This was obtained by determining the body calcium and phosphorus of a group of 6 rats of 28 days of age which were litter mates of the animals used in the experiment and substracting the values from those obtained for the experimental animals. The results are given in Table 12.

Discussion

It is evident from Table 10 that there is not much difference in the growth of rats fed diets containing crude, refined or hydrogenated cottonseed oil, refined groundnut oil or cow's ghee. Schantz *et al.*[37] reported that young rats grew best over the first six weeks after weaning on a diet of skimmed milk homogenized with butter fat. When corn oil, coconut oil, cottonseed oil or soyabean oil was used in place of butter fat, the growth was markedly less. The available evidence would show that the daily requirement of linoleic acid which is essential in nutrition is exceedingly small[38], being of the order of 20–50 mg. According to the data available, linoleic acid is present in adequate quantities in cow's ghee and groundnut oil[39]. Even though crude and refined cottonseed oils are richer in linoleic acid glycerides than cow's ghee and groundnut oil, still no significant difference in their growth-promoting value has been noted. The results obtained in the present investigation are in conformity with the observations of Deuel *et al.* [26] [27] who found no significant difference in the growth-promoting value of butter fat and soyabean oil even though the latter was richer than butter fat in linoleic acid glycerides.

The results of the metabolism and carcass analyses (Tables 3 and 4) do not show any significant difference in the effect of different fats on the utilization of calcium and phosphorus. The rats fed cottonseed vanas-

TABLE 12—STORAGE OF CALCIUM AND PHOSPHORUS IN RATS FED ON COTTONSEED OILS

(*Duration of experiment, 6 weeks*)

	Crude cotton-seed oil	Refined cotton-seed oil	Cottonseed vanaspati	Refined ground-nut oil	Cow's ghee
No. of rats	6	6	6	6	6
Av. initial wt., g.	52.1	52.1	52.1	51.9	52.0
Av. final wt., g.	144.2	145.0	138.1	138.3	147.9
Av. gain in weight, g.	92.1	92.9	86.1	86.4	95.9
Total food intake (dry), g.	358.7	362.5	356.2	346.3	358.6
Total Ca intake, mg.	1095.0	1107.0	1087.0	1057.0	1094.0
Initial body Ca, mg.	353.1	353.1	353.1	353.1	353.1
Final body Ca, mg.	1092.4	1144.2	1140.1	1052.9	1193.4
Ca retained, mg.	739.3	791.1	787.0	699.8	840.3
Ca utilized, %*	67.5 ± 2.4	71.5 ± 2.8	72.4 ± 2.6	66.2 ± 2.4	76.8 ± 2.7
Total P intake, mg.	1148.0	1159.0	1140.0	1108.0	1147.0
Initial body P, mg.	283.1	283.1	283.1	283.1	283.1
Final body P, mg.	1001.7	1041.1	891.8	883.4	952.8
P retained, mg.	718.6	758.0	608.7	600.3	669.7
P utilized, %*	62.6 ± 2.1	65.4 ± 3.4	53.4 ± 2.6	54.2 ± 2.1	58.4 ± 2.9

*Including standard error of the mean

TABLE 13—CHARACTERISTICS OF REFINED COTTONSEED OIL

	Present investigation	Range reported in literature
Sp. gr.	0.921	0.921 – 0.925
Ref. index	1.470	1.467 – 1.472
Sap. value	194.5	192.0 – 198.0
Iod. value	102.6	102.0 – 114.0
SCN value	60.3	60.0 – 70.0
Unsaponifiables, %	0.75	0.6 – 0.9
Sat. acids, %	34.0	22.0 – 36.0

TABLE 14—GLYCERIDE COMPOSITION OF REFINED COTTONSEED OIL

Glyceride	Present investigation	Range reported in literature
Saturated	34.9	22.5 – 36.2
Unsaturated	65.8	62.8 – 77.5
Oleic	18.2	18.0 – 30.0
Linoleic	47.6	40.0 – 35.0

pati diet excreted more of calcium in the feces than the other rats. The slightly higher degree of calcium utilization observed in the case of refined cottonseed oil and cow's ghee, may be due to higher content of oleic and linoleic acids which help in the absorption of calcium and phosphorus.

4. STABILITY OF CRUDE AND REFINED COTTONSEED OIL

In an earlier paper[16] from this laboratory, the results of studies on the composition of nine varieties of Indian cottonseed and of crude, refined and refined & bleached cottonseed oils have been reported. The present paper deals with investigations on the stability of cottonseed oil stored in metallic containers.

EXPERIMENTAL

The bulk sample of crude cottonseed oil used for the investigation was supplied by *Hindustan Vanaspati Manufacturing Co.* Crude cottonseed oil was refined and bleached according to the procedure described earlier by Narayana Rao and Kuppuswamy[16]. The official A.O.C.S. methods[4] were used in determining the physical and chemical characteristics of the oil. Saturated fatty acids were determined by the Bertram oxidation method as modified by Pelikan and Mikush[40]. The glyceride composition of the oil was calculated from thiocyanogen and iodine values. The results of these determinations are given in Tables 13 and 14 along with the range of values previously reported in literature.

Keeping quality: Crude, refined and bleached samples of cottonseed oil were kept in the dark, stored in glass, aluminium and brass vessels tinned inside, at room temperature (25°–30°C.) and at 37°C. Such a procedure was adopted as edible oils are usually stored in households in aluminium or brass vessels. Groundnut oil was also included in the study for comparison. The rate of development of peroxide value in the different oils was followed for a period of three months. The peroxide value was determined at intervals of 15 days according to the procedure of Wheeler[41] and expressed as milli-equivalents of thio. per kg. of fat. The results are given in Tables 15 and 16.

DISCUSSION

It is evident from the data presented in Table 15 that crude cottonseed oil kept well during three months' storage in glass, aluminium or brass

TABLE 15—PEROXIDE VALUE* OF DIFFERENT FATS DURING STORAGE

(*Initial peroxide value, 0*)

Period *days*	Crude cottonseed oil			Refined cottonseed oil			Refined & bleached cottonseed oil			Crude groundnut oil		
	Glass	Alumi-nium	Brass	Glass	Alumi-nium	Brass	Glass	Alumi-nium	Brass	Glass	Alumi-nium	Brass
						At 25°–30°						
15	0	0	0	8.0	19.0	21.0	8.0	26.0	24.0	0	2.1	2.1
30	0	0.2	0.3	15.0	35.0	36.0	18.0	36.0	32.0	1.2	6.0	5.6
45	0.2	0.6	0.6	26.2	66.0	65.5	32.4	44.0	39.5	1.8	7.6	6.5
60	0.4	1.2	1.2	30.5	98.0	94.6	44.5	76.8	62.6	2.4	12.2	9.6
75	0.9	1.9	1.8	38.5	139.0	141.5	52.6	103.5	82.0	2.6	15.2	11.6
90	1.5	2.2	2.5	51.0	125.0	126.0	62.0	148.0	139.0	2.9	16.0	14.0
						At 37°						
15	0	0	0.2	52.5	81.5	83.5	58.0	70.0	65.0	2.4	4.0	4.5
30	0.5	0.8	1.0	81.6	102.1	135.6	89.6	129.0	132.0	5.0	6.5	6.8
45	0.8	1.2	1.5	106.0	137.5	178.0	120.4	186.5	186.0	7.5	8.0	8.2
60	1.2	1.9	2.3	126.0	158.6	191.6	143.5	223.0	235.0	10.0	12.0	13.0
75	1.8	2.4	2.9	145.0	220.0	225.6	152.0	272.0	273.0	12.0	15.0	18.0
90	2.8	3.6	3.4	165.0	225.0	230.0	175.0	242.0	245.0	15.0	22.0	25.0

*ml. of N thio./kg. of fat

TABLE 16—EFFECT OF ANTIOXIDANTS ON DEVELOPMENT OF PEROXIDES*

(*Initial peroxide value, 0*)

Period *days*	With 0.02% BHA						With 0.02% dodecyl gallate					
	Refined cottonseed oil			Refined & bleached cottonseed oil			Refined cottonseed oil			Refined & bleached cottonseed oil		
	Glass	Aluminium	Brass	Glass	Aluminium	Brass	Glass	Aluminium	Brass	Glass	Aluminium	Brass
						At 25°–30°						
15	0.0	8.0	10.0	0	9.5	12.0	0	12.0	13.5	0	8.0	9.5
30	0.1	20.0	25.5	0.5	19.5	25.6	0.2	28.6	32.4	0.3	19.6	20.5
45	0.8	35.5	42.2	0.8	38.6	40.0	0.8	45.8	52.6	0.8	32.5	36·6
60	1.2	52.5	63.5	1.6	62.5	65.5	1.5	69.6	75.6	1.6	56.0	52.8
75	1.8	74.0	82.6	1.9	84.0	82.5	2.2	83.4	98.6	2.4	84.5	82.5
90	2.6	86.5	101.6	3.2	92.5	105.0	2.8	96.6	98.9	3.2	95.0	105.6
						At 37°						
15	0	38.5	36.0	0.2	50.0	52.0	0	38.4	39.5	0.1	50.6	50.2
30	1.2	78.6	77.0	1.3	79.5	78.0	1.8	75.6	79.6	1.3	82.0	85.0
45	2.4	120.4	125.5	2.4	120.6	125.5	3.0	122.5	130.5	3.2	136.0	125.0
60	3.6	167.0	172.5	4.2	180.5	192.5	3.8	165.0	164.2	4.6	167.8	153.5
75	4.8	186.0	192.0	5.8	200.5	215.0	4.9	192.5	186.5	5.4	199.8	177.5
90	5.4	178.2	186.6	7.0	192.0	198.0	6.2	182.3	183.6	7.5	200.0	195.0

*ml. of N thio./kg. of fat

containers. The stability of the crude oil to oxidative rancidity is due to the presence of gossypol (conc. in oil, 0.4%) which possesses antioxidant properties. The crude oil, even when stored in metallic containers, did not develop rancidity to an appreciable extent. This is probably due to the fact that it contains appreciable amounts of lecithin and other phosphatides which are known to be metal deactivators. Refined cottonseed oil, however, did not keep well and developed peroxides rapidly. The peroxide value of refined and refined & bleached cottonseed oil stored in aluminium and brass containers was higher than that of the oil stored in glass containers. This is due to the pro-oxidant effect of metals. Refined and refined & bleached cottonseed oil to which antioxidants had been added kept well when stored in glass containers. But when stored in metal containers, they developed peroxides quite rapidly. This shows that the antioxidants used did not protect the oils against the development of oxidative rancidity catalysed by metals.

5. STABILITY OF WINTERIZED COTTONSEED OIL

In the preparation of some foods, the consistency of the oil is a matter of great importance. For example, an oil which does not solidify at 0–4°C. should be used for preparing green salads. In the U.S.A., considerable quantities of salad oil prepared from winterized cottonseed oil are utilized for the manufacture of mayonnaise and salad dressings[5]. The composition and keeping quality of the winterized oil depends on the conditions of winterization. Apart from the recent work of Mack, Moore and Bickford[42] on the chemical composition and autoxidative stability of three samples of winterized cottonseed oil by the active oxygen method, no information is available regarding the keeping quality of winterized cottonseed oil. The present paper deals with studies on the keeping quality of winterized cottonseed oil stored in glass and tin containers, with and without the addition of antioxidants and synergists.

Experimental

Preparation of winterized oil: A sample of crude cottonseed oil was refined and given a light bleach according to the procedure described by Narayana Rao and Kuppuswamy[16]. The bleached oil was kept in a cold chamber at 4.4-6.1°C. for a period of 5 days, during which solid stearine fraction separated out. The winterized oil was decanted and filtered at the same temperature. The yield of winterized oil was 76 per cent of the refined & bleached oil.

Characteristics of the oil: The physical and chemical constants and cold test of the winterized oil as also of the original refined cottonseed oil were determined according to the standard A.O.C.S. methods [4]. The total tocopherol content of both winterized and unwinterized cottonseed oil was estimated according to Emmerie and Engel's method as modified by Parker and McFalane[43]. The results of these determinations are presented in Table 17.

Keeping quality: Samples of winterized cottonseed oil and refined & bleached cottonseed oil were stored in dark in glass bottles and tin cans

at room temperature (23–30°C.) and at 37°C. In order to study the effect of certain antioxidants and synergists on the keeping quality of the oils, dodecyl gallate and propyl gallate (0.02 per cent) and synergist, citric acid (0.1 per cent), were added. Samples containing antioxidants and synergist were stored under the same conditions as control samples. The peroxide values were determined at intervals of 15 days during a period of three months according to the procedure of Wheeler [41]. The results are given in Table 18.

Discussion

Table 17 shows that winterization did not appreciably affect the colour of refined cottonseed oil. A slight increase in iodine value, glycerides of oleic and linoleic acids and tocopherol content, and a decrease in the saturated glycerides were noted in the winterized oil.

The data in Table 18 show that the keeping quality of winterized oil is nearly of the same order as that of the unwinterized oil in spite of the slightly higher iodine value of the former. Similar observations have been made by Mack *et al.*[42] Both oils developed peroxides more rapidly at room temperature (23–30°C). Oils stored in tin vessels developed slightly higher peroxide values than those stored in glass. The development of higher peroxide values in vegetable oils when stored in tin containers as compared to glass has been observed by other workers[44 45]. The two esters of gallic acid (propyl gallate and dodecyl gallate) in the presence of citric acid were effective in retarding oxidative rancidity in unwinterized and winterized cottonseed oils stored in glass and tin containers.

Table 17—Characteristics of Refined & Bleached and Winterized Cottonseed Oils

	Refined and bleached oil		Winterized oil	
	Present investigation	Values reported in literature	Present investigation	Values reported in literature
Colour*	12.5 Y:1.1R	35–70 Y:3.2–6R	12.0 Y:1.0R	35–70 Y:3.2–4.1R
Iodine value	105.3	102–114	109.2	103–116.8
Thiocyanogen value	65.8	64–69	68.1	67–71
Linolein, %	48.8	47–51	50.8	49–55
Olein, %	24.2	19–21	25.0	22–25
Saturated glycerides, %	27.0	23–27	24.2	19–29
Total tocopherols, mg. %	75	60–120	87	75–150
Cold test, hr.	..	..	8	..

*Lovibond units, 1 cm. cell

TABLE 18—EFFECT OF ANTIOXIDANTS AND SYNERGIST ON PEROXIDE* DEVELOPMENT IN COTTONSEED OILS

Period of storage *days*	Refined & bleached oil						Winterized oil					
	Control		D.G. + C.A.		P.G. + C.A.		Control		D.G. + C.A.		P.G. + C.A.	
	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
						At 23°–30°C.						
0	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
15	14.0	15.0	7.5	7.5	7.5	8.0	14.5	15.5	7.5	8.0	7.5	8.5
30	23.0	25.0	8.0	8.5	8.5	8.5	22.5	25.5	8.5	9.0	8.5	9.0
45	33.0	36.5	8.5	10.0	9.0	9.5	34.0	38.0	8.5	11.0	9.5	9.5
60	45.0	50.5	9.5	11.5	10.0	11.0	46.0	53.0	9.5	12.5	10.0	12.0
75	60.0	57.5	10.0	13.0	10.5	12.0	62.5	69.5	10.5	14.0	11.0	13.5
90	80.0	89.5	11.0	15.0	11.0	14.0	83.5	93.5	11.5	16.0	12.5	15.0
						At 37°C.						
0	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
15	20.0	22.0	8.5	9.0	8.0	8.5	21.5	23.0	8.5	9.0	8.0	8.5
30	41.0	44.5	11.0	11.0	10.5	11.0	42.5	46.0	11.5	11.5	10.5	11.0
45	70.5	79.5	12.0	13.5	11.0	13.5	73.0	80.0	12.5	14.5	11.5	14.0
60	109.0	124.0	14.5	16.5	12.5	15.0	112.5	126.0	15.5	17.0	13.5	16.0
75	148.5	163.5	16.0	19.0	14.0	17.0	125.0	166.5	17.0	20.5	15.5	18.5
90	195.0	209.5	18.0	22.0	16.0	19.5	200.0	214.5	19.0	23.5	17.5	21.5

*ml. of 1 N thio./kg. of fat; D.G.—Dodecyl gallate ; C.A.—Citric acid ; P.G.—Propyl gallate

TABLE 19—BLEACHING PROPERTIES OF ACTIVATED EARTHS

[*Particle size, > 100 mesh; activated earth, 4% (w/w); colour index of untreated oil: 2.2Y*]

Treatment	Bagheshapura clay		Hebbur clay		Nandhihalli clay		Thirthahalli clay	
	Colour index*	Bleach obtd., %	Colour index *	Bleach obtd., %	Colour index*	Bleach obtd., %	Colour index*	Bleach obtd., %
Heated to 300–400°C.	1.3	41.0	1.5	31.8	2.1	4.6	2.0	9.1
Heated o 350–400°C.	1.3	41.0	1.5	31.8	2.1	4.6	2.0	9.1
Washed free of salts and dried at 110°C.	1.6	27.3	1.7	22.7	2.0	9.1	1.8	18.2
Treated with 5N HCl. washed and dried at 110°C.	1.2	45.5	1.3	41.0	1.7	2.7	1.6	27.3
Same as 4, but heated to 350°C.	1.2	45.5	1.3	41.0	1.7	22.7	1.6	27.3
Treated with conc. HCl washed and dried at 110°C.	1.3	41.0	1.2	45.5	1.7	22.7	1.6	27.3
Same as 6, but heated to 350°C.	1.3	41.0	1.2	45.5	1.7	22.7	1.6	27.3

*Lovibond scale, yellow units, 1 cm. cell

6. PREPARATION OF ACTIVATED CLAYS AND CARBONS FROM INDIGENOUS RAW MATERIALS FOR BLEACHING COTTONSEED OIL

Fuller's earth and bleaching carbon are largely used in the oil industry for clarification and decolorisation. Owing to increased production of hydrogenated oil in the country, the demand for bleaching earths and bleaching carbons is on the increase. The use of imported varieties of bleaching earths and bleaching carbons increases the cost of bleaching considerably. Consequently, several workers[46–51] have investigated the suitability of activated indigenous clays and bleaching carbons prepared from cheap raw materials for decolorisation of oils and fats.

Activation of Indigenous Earths

Four kaolinic clays, namely, Bagheshapura, Hebbur, Nandhihalli and Thirthahalli clays, were obtained from the Bureau of Mineral Development, Mysore, powdered to pass through a 100 mesh sieve, and then activated according to the procedure of Joshi and Saletore [49]. Comparative tests were carried out with these clays and three well known foreign earths, namely B.D.H. fuller's earth, fuller's earth obtained from *Hindustan Vanaspati Manufacturing Co.*, and Tonsil, an activated German earth. The bleaching trials were made at 4 per cent level on refined groundnut oil, according to the A.O.C.S. methods[4]. The oils were filtered hot and the filtrate collected after discarding the first few ml. The intensity of yellow colour was measured in a Lovibond tintometer using a 1 cm. cell. The results are given in Tables 19 and 20.

It is evident from the results that Bagheshapura clay, when heated to a high temperature (300–350°C.), gives an activated product with improved bleaching action. Activation with 5 N hydrochloric acid gives a better product. Hebbur clay also gives a product with good bleaching activity when activated wih 5 N hydrochloric acid.

Nandhihalli and Thirthahalli clays do not give activated products with good bleaching activity. Foreign earths, which were used for comparison, were found to have finer particle size (>200 mesh) than

Table 20—Bleaching Properties of Foreign Earths as compared to Bagheshapura Clay

(*Particle size*, >200; *activated earths*, 4% (*w/w*); *colour index of untreated oil*, 2.2 Y)

Earth	Colour index*	Bleach %
B.D.H. fuller's earth	1.1	50.0
H.V.M. fuller's earth	1.0	54.5
Tonsil	1.1	50.0
Tonsil (Optimum)	1.1	50.0
Bagheshapura clay	1.1	50.0

*Lovibond yellow units, 1 cm. cell

TABLE 21—BLEACHING PROPERTIES OF ACTIVATED CARBONS

[*Particle size, >125 mesh; % (w/w) added to oil, 0.5; colour index of untreated oil, 3.3 R; 26 Y*]

Raw material		Activator	Colour index* and bleach			
			Red	Bleach %	Yellow	Bleach %
Saw dust†	(i)	Zinc chloride	1.5	54.5	16.5	35.5
do	(ii)	do	1.8	45.5	18.0	30.8
do	(iii)	do	2.3	30.3	17.0	34.6
do	(iv)	do	2.2	33.3	18.0	30.8
do	(v)	do	2.3	30.3	18.0	30.8
Saw dust	(i)	Calcium oxide	1.8	45.5	15.0	42.3
do	(ii)	do	1.4	57.6	15.0	42.3
do	(iii)	do	1.4	57.6	14.0	46.1
do	(iv)	do	2.1	36.4	18.0	30.8
do	(v)	do	2.3	30.3	20.0	23.0
Paddy husk		Zinc chloride	1.0	70.0	12.0	54.0
		Calcium oxide	2.7	18.2	22.0	15.4
Groundnut hulls		Zinc chloride	1.2	63.6	13.0	50.0
		Calcium oxide	2.3	30.3	18.0	30.8
Cottonseed hulls		Zinc chloride	2.3	30.3	19.0	26.9
		Calcium oxide	2.3	30.3	22.0	15.4
I.C.I. carbon			1.5	54.5	17.0	34.6
Penick carbon			1.1	66.7	12.0	54.0

*Lovibond units, 1 cm. cell

†(i) *Honne* (ii) Teak (iii) Silver oak (iv) *Burga* (v) *Tadasil*

the products prepared by us. Hence, it is expected that the bleaching properties of the activated Mysore clays may further improve if ground to a finer particle size (>200 mesh). A sample of acid activated Bagheshapura clay with fine particle size (>200 mesh) was found to be as active as B.D.H. fuller's earth in its bleaching action (Table 20).

BLEACHING CARBONS

Bleaching carbons were prepared from indigenous saw dusts (teak, *Honne*, *Tadasil*, *Burga*, silver oak), groundnut hulls, cottonseed hulls and paddy husk, after activation with zinc chloride and calcium oxide, and

TABLE 22—BLEACHING EFFICIENCY OF ACTIVATED EARTHS AND CARBONS AND THEIR MIXTURES

(Colour of untreated oil, 5.5 R; 30 Y)

Bleaching agent	Colour of bleached oil*		Bleach, %	
	Red	Yellow	Red	Yellow
Paddy husk carbon 4%	1.3	9.5	76.3	68.3
do 2%	1.5	12.0	72.7	60.0
do 1.0%	1.9	14.0	65.4	53.3
do 0.5%	2.4	16.5	56.4	45.0
I.C.I. carbon 4%	1.9	12.4	65.4	58.6
do 2%	2.5	17.6	54.5	41.3
do 1.0%	2.8	20.8	49.1	30.6
do 0.5%	3.7	22.4	32.7	25.3
Activated Bagheshapura clay 6%	1.4	10.4	74.5	65.3
do 4%	2.5	14.0	54.5	53.3
do 2.5%	3.0	18.0	45.4	40.0
do 1.0%	4.3	24.0	21.8	20.0
Fuller's earth 6.0%	1.2	9.8	78.2	67.3
do 4.0%	1.2	12.5	60.0	58.3
do 2.5%	2.5	15.0	54.5	50.0
do 1.0%	3.7	21.0	32.7	30.0
Paddy husk carbon & Bagheshapura clay (1:10) 4.4%	1.1	7.5	80.0	75.0
do 3.3%	1.3	12.0	76.3	60.0
do 2.2%	1.8	15.5	67.2	48.3
do 1.1%	2.9	21.3	47.3	29.0
I.C.A. carbon & fuller's earth (1:10) 4.4	1.0	7.0	81.8	60.6
do 3.3	1.2	11.5	78.2	61.6
do 2.2	1.6	13.0	70.8	56.6
do 1.1	2.5	20.0	54.5	33.3

*Lovibond units, 1 cm. cell

their bleaching activity was compared with two imported carbons (Penick and I.C.I. carbon).

All the raw materials were ground to pass through a 40 mesh sieve and activated with zinc chloride and calcium oxide. The procedure for the activation with zinc chloride was the same as that described by Mukherjee and Bhattacharya[50]. In the case of paddy husk, the material was digested with sodium hydroxide and then activated wih zinc chloride. The method adopted for the activation with calcium oxide was similar to that with zinc chloride with the exception that an equal quantity of calcium oxide was used in place of zinc chloride.

The relative efficacy of the different carbons in bleaching cottonseed oil was determined according to the methods of A.O.C.S.[4] The colour of the bleached oil was measured in Lovibond tintometer using a 1 cm. cell. The results are given in Table 21.

The results (Table 21) show that paddy husk carbon after activation with sodium hydroxide and zinc chloride was efficient in bleaching refined cottonseed oil. The carbon prepared from groundnut hulls, after activation with zinc chloride, also possessed high bleaching activity and compared well with imported carbon.

Bleaching Efficiency of Activated Clays and Carbons

The bleaching efficiency of paddy husk carbon, acid activated Bagheshapura clay and a mixture of paddy husk carbon and Bagheshapura clay (1 :10) at various percentages was determined according to the methods of A.O.C.S.[4] The colour of bleached oil was measured in a Lovibond tintometer using a 1 cm. cell. The results, as compared with those obtained with I.C.I. carbon, imported fuller's earth and mixture of I.C.I. carbon and fuller's earth are presented in Table 4.

It is evident from the results (Table 22) that paddy husk carbon and acid activated Bagheshapura clay as well as their mixtures compare favourably with foreign carbons and earths in bleaching refined cottonseed oil.

7. COMPOSITION, STORAGE AND NUTRITIVE VALUE OF PALM OIL

During recent years the production of hydrogenated vegetable oils has been on the increase and there is a growing demand for the oils suitable for hydrogenation. There is thus an urgent need to investigate the suitability of other sources of vegetable oils to supplement groundnut oil in the production of hydrogenated products.

Palm oil is the orange coloured oil derived from the outer pulp of the fruit of oil palm (*Elaeis guineensis* Jacq. and other allied species). Although the oil has been used for edible purposes for many years, little information is available about its keeping quality and nutritive value.

The present paper deals with the studies on the composition, stability and nutritive value of palm oil and a blend of palm oil and hydrogenated groundnut oil as compared to hydrogenated groundnut oil.

Experimental and Results

Characteristics and composition: Samples of palm oil, palm oil blend and hydrogenated groundnut oil used in the present investigation were supplied by *Hindustan Vanaspati Manufacturing Co. Ltd.*, Bombay.

The official methods of the A.O.C.S.[4] were used in determining the different physical and chemical characteristics of the oil. The melting point of the samples was determined by the V.O.P. method[52]. The iodine value was determined by the Hanus method[3]. The glyceride composition of the oils was calculated from the thiocyanogen and iodine values. The results are given in Table 23.

TABLE 23—CHEMICAL AND PHYSICAL CHARACTERISTICS OF PALM OIL AND BLENDS

Particulars	Palm oil*	Palm oil† blend	Hydrogenated groundnut oil
Consistency	Semi-solid‡	Solid (grainy)	Solid
Melting point, °C. (V.O.P. method)	31.5	36.5	36.5
Saponification value	202	198	196
Iodine value (Hanus method)	48.7	56.2	62.1
Thiocyanogen value	39.5	52.0	61.4
Olein, %	34.1	55.55	71.39
Linolein, %	11.2	4.86	0.48
Saturated glycerides, %	54.7	39.59	28.13
Carotene (microgram), %	260	30	nil
Initial free fatty acids, % oleic	0.21	0.08	0.05

*Refined and deodorized
†Palm oil blend (m.p., 36.5°C.), containing: hardened palm oil, 50% ; hardened groundnut oil, 45% ; and sesame oil, 5%
‡Separating into liquid and solid fractions

TABLE 24—PEROXIDE VALUES* OF FATS STORED WITH AND WITHOUT ANTIOXIDANTS AND SYNERGIST

(*Closed tin at 37°*)

Period *months*	Palm oil				Palm oil blend				Hydrogenated groundnut oil			
	Control	C.A.	BHA+C.A.	PG+C.A.	Control	C.A.	BHA+C.A.	PG+C.A.	Control	C.A.	BHA+C.A.	PG+C.A.
0	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	0	0	0	0
3	2.5	2.5	2	1	2.5	2.5	1	1	1.5	1	0	0
6	2.5	2.5	2.2	2.0	2.5	2.5	2.0	1.5	2.0	1.5	1	1

*ml. of 1 N thio./kg. of fat
C.A.—Citric acid; BHA—Butylated hydroxy anisole ; P.G.—Propyl gallate

TABLE 25—ACIDITY OF FATS STORED FOR 6 MONTHS AT 37°C.

Fat	Free fatty acids (% oleic acid)	
	Initial	Final
Palm oil (refined)	0.21	0.24
Palm oil blend and groundnut oil	0.08	0.10
Hydrogenated groundnut oil	0.06	0.06

TABLE 26—COMPOSITION OF EXPERIMENTAL DIETS

	%
Casein	12
Corn starch	60
Sugar	10
Vitaminised starch*	4
McCollum and Davis Salt Mixture	4
Fat†	10

*At 4% level, vitaminised starch satisfies the requirements of all B-group vitamins †Refined and deodorised palm oil, palm oil blend and hydrogenated groundnut oil

TABLE 27—GROWTH PROMOTING VALUE OF FATS

Fat	Av. food intake: per rat per day g.	Av. weekly gain in weight g.	
Palm oil	11.6	13.36	0.820 ± (16 d.f.)
Palm oil blend	11.5	13.88	
Hydrogenated groundnut oil	11.5	14.15	

Stability: The keeping quality of different fats was studied by storing them in 1 lb. closed tins at 37°C. for a period of six months and by following the acidity and peroxide values. The effect of certain antioxidants, e.g., butylated hydroxy anisole (0.02 per cent) and propyl gallate (0.01 per cent) along with synergist, citric acid (0.05 per cent) on the keeping quality of the different fats was also studied. The free fatty acid content of the control samples was determined at the beginning and at the end of the experimental period. The results are presented in Tables 24 and 25.

Nutritive value: For assessing the relative nutritive value of the three fats, the following experiments were carried out with albino rats.

(1) growth-promoting value when incorporated at 10 per cent level in a synthetic diet ;
(2) digestibility co-efficient ; and
(3) effect on calcium and phosphorus metabolism.

TABLE 28—DIGESTIBILITY COEFFICIENTS OF DIFFERENT FATS

(*Experimental period. 7 days*)

Fat	Digestibility coeff. %
Palm oil	96.6
Palm oil blend	96.8
Hydrogenated groundnut oil	96.1

TABLE 29—EFFECT OF FATS ON CALCIUM UTILIZATION

(*Experimental period, 6 days*)

Fat	Av. food intake *g.*	Av. Ca consumed *mg.*	Av. Ca excreted, *mg.*		Av. Ca balance *mg.*	
			Urine	Feces		
Palm oil	54.8	175.4	1.7	64.7	109.0	$\pm^{4.34}$ (8 d.f)
Palm oil blend	56.1	185.1	2.6	74.8	107.7	
Hydrogenated groundnut oil	63.5	203.2	3.6	91.0	108.6	

TABLE 30—EFFECT OF DIFFERENT FATS ON PHOSPHORUS UTILISATION

(*Experimental period, 6 days*)

Fat	Av. food intake *g.*	Av. P consumed *mg.*	Av. P excreted		Av. P balance *mg.*	
			Urine	Feces		
Palm oil	54.8	400.8	90.6	40.8	269.4	$\pm^{10.6}$ (8 d.f.)
Palm oil blend	56.1	410.0	108.0	45.8	256.2	
Hydrogenated groundnut oil	63.5	464.1	134.2	61.4	268.5	

TABLE 31—ORGANOLEPTIC EVALUATION OF DISHES CONTAINING DIFFERENT FATS

	Sweet (Mysore Pak)			Savoury (Bhajji)		
	Refined palm oil	Palm oil blend	Hydrogenated groundnut oil	Refined palm oil	Palm oil blend	Hydrogenated groundnut oil
Texture	Hard	Crisp	Crisp	Crisp	Crisp	Crisp
Flavour	Marked palm oil flavour	Slight palm oil flavour	Bland	Slight palm oil flavour	Bland	Bland
Acceptability	Fair	Good	Very good	Good	Very good	Very good

Growth-promoting value: Three groups of freshly weaned rats, about four weeks old and weighing between 40 and 45 g., were used in these studies. Each group contained ten rats, distributed equally according to sex and litter mates. The three groups of rats were fed *ad lib* on experimental diets containing palm oil, palm oil blend and hydrogenated groundnut oil. The composition of the experimental diet is shown in Table [26]. The fats were incorporated in the experimental diets at 10 per cent level. In addition to the diet, two drops each of Adexoline and alpha-tocopherol were given orally to each rat, twice a week, to meet vitamins A, D and E requirements. The feeding was carried out for a period of eight weeks. The average increase in weight of the rats is given in Table 27.

Digestibility: The digestibility coefficients of palm oil, palm oil blend and hydrogenated groundnut oil were determined on adult rats, by feeding them diets containing the different fats at 10 per cent level. The method adopted was similar to that of Cheng *et al.*[53] The composition of the diet was the same as that given in Table [26]. The digestibility coefficients were calculated in the usual manner after correction for metabolic fat. The results are given in Table 28.

Effect of fats on calcium and phosphorus metabolism: The effect of incorporating the fats at 10 per cent level in an adequate diet on the calcium and phosphorus metabolism was studied in growing albino rats. The composition of the diet was the same as that given in Table 4. Three groups of growing albino rats (about 6 weeks old, each rat weighing 80—85 g.) were used. Each group contained six rats (3 males and 3 females). The animals were fed diets containing the different fats. After a preliminary period of 5 days on experimental diets, the feces and urine were collected for a period of six days, adopting the methods followed by Narayana Rao and Swaminathan.[54] Calcium and phosphorus in urine, feces and food were estimated according to the methods followed by Murthy *et al.*[55] The results are given in Tables 29 and 30.

Consumer acceptability of dishes prepared using different fats: In order to assess the relative acceptability of the fats as cooking medium, one sweet dish (*Mysore Pak*) and one savoury dish (*Bhajji*) were prepared using the different fats. The consumer acceptability of preparations was evaluated by a panel of 12 judges selected from among the staff members of the Institute. The results of organoleptic evaluation of the fats are given in Table 31. The results show that the flavour of palm oil could be easily detected in the sweet dish made from palm oil, but not in that made from the blend. The different fats may be ranked in the following descending order regarding their acceptability in sweet preparations: hydrogenated groundnut oil; blend of hydrogenated groundnut oil and palm oil; and refined palm oil. There was no perceptible difference, however, in the flavour and acceptability of the savoury dish prepared using the three different fats.

Discussion

Palm oil has a semi-solid consistency, separating into liquid and solid fractions even at room temperature. The results in Table 23 show that it is a rich source of carotene (260μ/100 g.). The results on the keeping quality of different oils (Tables 24 and 25) show that palm oil is fairly stable, compar-

ing favourably in this respect with the blend as well as hydrogenated groundnut oil. Addition of citric acid alone has no stabilising effect on the oils. Addition of butylated hydroxy anisole and propyl gallate along with citric acid improves the keeping quality of different fats. No appreciable increase in the acidity of oils due to storage for a period of six months was observed.

The results (Table 26) on the nutritive value of the oils show that there is no significant difference in their growth-promoting value. Previous workers[26,27,56] also could not observe any significant difference in the growth-promoting value of different vegetable and animal fats. The three fats, palm oil, palm oil blend and hydrogenated groundnut oil, were almost completely digestible. Deuel *et al.*[27] observed that fats which have a melting point below 50°C. are almost completely digested by rats. No significant difference was observed in the retention of calcium and phosphorus in rats fed palm oil, palm oil blend and hydrogenated groundnut oil.

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Summary

Nine Indian varieties of cottonseed were examined for their linter, hull and kernel composition ; physical and chemical characteristics of oil obtained from them and methods of refining and bleaching the oil.

The proportion of linter, hull and kernel in Indian varieties was slightly lower than those reported for American varieties.

The oil contents of whole seeds and kernels ranged from 14 to 19.4 per cent and 29 to 35 per cent respectively (slightly lower than those reported for American varieties).

Alkali refining of crude cottonseed oil yielded an oil with light colour (Lovibond units : 10.0 R ; 19.9 Y). Re-refining improved the colour to an appreciable extent and bleaching of re-refined oil with a mixture of fuller's earth and sawdust charcoal gave an oil with very little colour (Lovibond units : R 0.8; Y 3.8). Fuller's earth alone had some bleaching effect, but along with animal charcoal or sawdust charcoal, it served as a good bleaching agent.

The gossypol content of Indian cottonseed varied from 0.7–0.92 per cent. Refining of cottonseed oil completely removed gossypol from the oil.

In vitro digestibility of crude, refined and hydrogenated cottonseed oils, refined groundnut oil and cow's ghee has been studied using castor and pancreatic lipases.

Of all the fats, cow's ghee was most rapidly digested, the rate of digestion of the other fats being nearly the same. The digestibility co-efficient of these fats, as judged by feeding trials on adult albino rats, was of the order of 95 per cent.

Nutritive value of crude, refined and hydrogenated cottonseed oils, groundnut oil and cow's ghee has been studied.

There was no significant difference in the growth-promoting value of these fats at 10 per cent level in adequate synthetic diets in young albino rats.

Body storage and metabolism studies on the effect of these fats on calcium and phosphorus metabolism showed that rats fed hydrogenated cottonseed oil diet excreted more of calcium in the feces than the other rats. The percentage of calcium utilized by rats fed diets containing cottonseed oil and cow's ghee was slightly more than that utilised by rats fed on other fats.

The stability of crude, refined and refined & bleached cottonseed oil when stored in glass, aluminium and tinned brass containers for a period of three months, as judged by changes in peroxide value, has been examined.

Crude cottonseed oil was stable when stored in glass containers. While the oil was stable even when stored in metallic containers, refined cottonseed oil was not stable.

Addition of butylated hydroxy anisole (BHA) and dodecyl gallate at 0.02 per cent level to refined and refined & bleached cottonseed oil stored in metal containers did not prevent the development of peroxides.

The physical and chemical constants of winterized cottonseed oil as well as of refined & bleached cottonseed oil have been determined.

The autoxidative stability of cottonseed oil was not materially affected by winterization. Propyl and dodecyl gallates along with citric acid as synergist were effective in retarding the development of peroxides.

The bleaching properties of four activated Mysore clays and of activated vegetable carbons from cheap raw materials like saw dust, groundnut and cottonseed hulls and paddy husk have been tested and compared with some imported earths and carbons.

Tests using refined groundnut and cottonseed oils showed that Baghershapura clay was the best and was as efficient as certain imported earths.

Similar tests with activated carbons using refined cottonseed oil showed that some of the carbons prepared were as efficient as imported carbons.

The physical and chemical constants and carotene content of palm oil, palm oil blend, and hydrogenated groundnut oil have been determined.

The keeping quality of palm oil and palm oil blend was nearly the same as that of hydrogenated groundnut oil.

Addition of butylated hydroxy anisole (0.02 per cent) and propyl gallate (0.01 percent) along with citric acid (0.005 per cent) enhanced the keeping quality of the fats.

There was no significant difference in the growth-promoting value of the three fats. Rats were able to digest all the three fats almost completely. There was no significant difference in the amount of calcium and phosphorus retained by different groups of rats fed diets containing the three fats.

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